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[GANN, 49, 1~4; March, 1958]

HISTOLOGICAL CHANGES IN TUMOR TISSUE INDUCED BY IONISING RADIATION 1. ANIMAL EXPERIMENT

(Plate I)

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Introduction

In radiation therapy of malignant tumor, the purpose of treatment is achieved by killing every malignant cell. In order to give such lethal action to the tumor, in every case, radiotherapists have to know that lethal tumor dose of radiation should be given to the patients. The determination of lethal tumor dose in every case is not possible without confirmation of lethal effect on tumor tissue. But the lethal effect does not appear completely at once after irradiation; there is a certain latent period, and the histological change in tumor tissue due to irradiation gradually arrives to its maximum point after a lapse of time. Therefore in discussing the lethal dose, we should first study when lethal effect will arrive to its maximum point after irradiation. In this paper, we report on the results of our experiments on the latent period, in the sense mentioned above, along the line of A. Glucksmann's research in 1946.

Experimental Material and Methods

1. Material. In this experiment, rats of about 100 g body weight were used. 0.5 cc of ascites of transplantable Yoshida Sarcoma was injected into the subcutaneous tissue of the left thigh of each rat. Transplanted tumors gradually grew up to 2.5 cm in diameter in all of the cases in 5 days after implantation and these subcutaneous tumors were used as material.

2. Method. In this experiment, the time as long as about 30 hours was required for irradiation. Accordingly, in order to eliminate both the fatigue by the restriction and the effect by whole-body irradiation of animals. We designed a new method for irradiation, as shown in Figure 1. Owing to this method, animals were able to get their food and their bodies were always protected from radiation while their tumors were being irradiated.

All animals with tumor were divided into 5 groups. Each group consisted of 5 animals. One of the groups was separated and was not irradiated. Other 4 groups were irradiated by radium.

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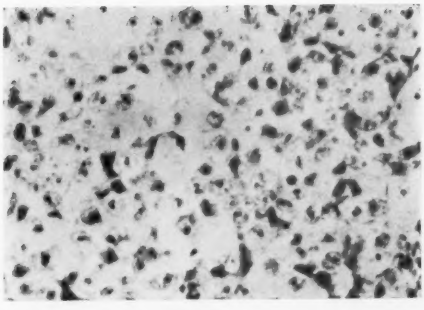
要　旨

放射線による腫瘍組織の組織細胞学的変化　1. 動物実験

塚本憲甫，北川俊夫
(癌研究所附属病院放射線科)

ラッテの大腿部皮下に移植した吉田肉腫に，ラジゥムを用いて長時間連続照射を行い，照射後摘出した腫瘍組織の活動性腫瘍細胞数を時間の経過に従って Glucksmann の方法により算定し，一曲線を得た。この曲線より，照射後，腫瘍の致死的効果が充分現われるのに少くとも3週日を要することが分った。

すなわち放射線照射後，その腫瘍に対する致死的効果を論ずるためには，少くとも3週日以上経過していなくてはならないことを知った。



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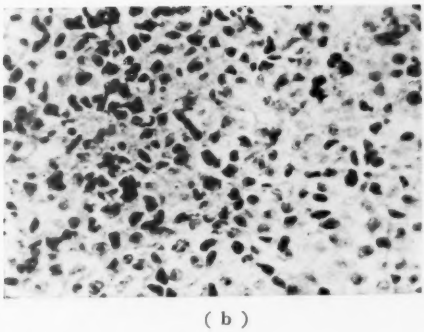

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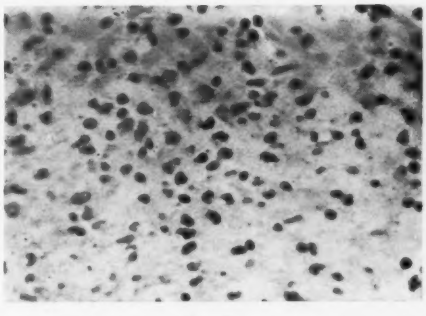

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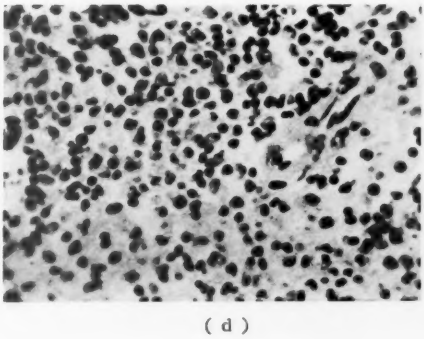

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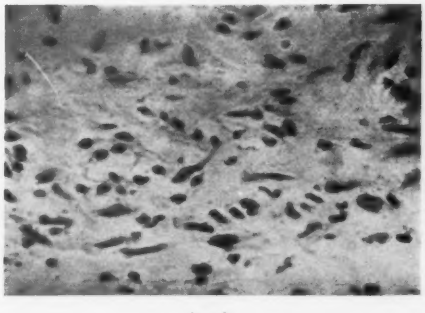

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[GANN, 49, 5~8; March, 1958]

HISTOLOGICAL CHANGES IN TUMOR TISSUE INDUCED BY IONISING RADIATION 2. CLINICAL EXPERIMENT

(Plates II–X)

KEMPO TSUKAMOTO and TOSHIO KITAGAWA
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INTRODUCTION

In our animal experiment, the lethal effect was confirmed to reach the maximum point at the third week after irradiation. This experiment has been performed by a single exposure to radium.

However, in practical radiation therapy of human malignant tumor, fractional irradiation is commonly used. It is sure that there is some difference of period in the appearance of lethal effect between single exposure and fractional irradiation. In this paper, we treated some cases of human cancer with various methods of irradiation and observed their histological change in the same way as in our animal experiment.

EXPERIMENT A

Before studying the lethal effect for human cancer, we should try again the possibility of adapting Glucksmann's method for every human cancer. The experiment was carried out for that purpose.

Method of Experiment. In Experiment A, various kinds of cancer were used as material and were treated with various methods of irradiation.

Biopsy was taken before treatment for control. Other biopsies were taken out during and after the treatment at intervals of several days. The number of viable tumor cells was counted in the same way as in the animal experiment. A few of these cases are shown, as follows.

Case 1. Male, aged 47. (Plate II)

Carcinoma of the penis; (Squamous cell carcinoma). Fractional external irradiation by radium; intensity; 44.2/hr., total tumor dose; 5707 r.

In Plate the thick line, along the horizontal axis, indicates over-all time of irradiation. In this figure, a curve descends rapidly to point b, after that, the curve changes slowly, the number of viable tumor cells is 0 at both points c and d.

This case has not indicated tumor recurrence for 3 years after treatment.

Case 2. Female, aged 51. (Plate III)

Carcinoma of the breast (Adenocarcinoma). Fractional irradiation by X-ray; total tumor dose; 6548 r.

As shown in Plate III, the number of viable cells is 0 both at points c and d. This case has shown primary cure.

Case 3. Female, aged 51. (Plate IV).

Carcinoma of the breast (Adenocarcinoma). Fractional external irradiation by radium; intensity: 50 r/hr.; total tumor dose; 4650 r.

As seen in Plate IV the curve that descends to point b ascends rapidly again to point c.

In this case, tumor showed local recurrence and many metastases occured. After several months, this patient gradually become cachectic and died.

Case 4. Female, aged 57 (Plate V).

Carcinoma of the tongue (Squamous cell carcinoma). Continuous irradiation by radium; intensity: 542 r/hr.; total tumor dose: 2500 r.

In Plate V, the curve, after point b, ascends rapidly again to point c. Tumor showed recurrence clinically and the patient died before long.

Case 5. Male, aged 48 (Plate VI).

Carcinoma of the parotis (Mucoepidermoid carcinoma). Fractional irradiation by teleradium at 2.0 gr.; total tumor dose: 2310 r.

The change of curve is relatively slow due to the fact that over-all time was too long. In Plate VI, the curve ascends to point c as soon as irradiation was finished. In this case, tumor showed recurrence definitely after one month, and the patient died.

Case 6. Male, aged 41 (Plate VII).

Carcinoma of the maxillary sinus (Squamous cell carcinoma). Fractional irradiation by X-ray; total tumor dose: 5400 r.

In Plate VII, the curve descends relatively rapidly at first and reaches nearly 0 at point b but the curve ascends again to point c in a little while. Tumor also showed its recurrence clinically.

Experimental Result. The result of this experiment proved that the tendencies shown by these curves agreed well with their clinical courses. In other words, Glucksmann's study was found to fit not only into the outcome of animal experiment but also into clinical courses. The definition of viable tumor cell, as we have mentioned in the animal experiment, is adapted for those clinical experiments too.

Experiment B

The result of Experiment A indicates the possibility of using the method of cell counting in the clinical experiment. But we can not investigate directly the

lethal effect in clinical experiment, because whole part of tumor is not taken out but only a part is taken at a time, so it is necessary to investigate the period for the appearance of tumor recurrence, according to the clinical course.

Method of experiment. 19 cases of skin cancer were used. (All cases had tumor recurrence). The histological characters of all were squamous cell carcinoma. This experiment was done in the same way as Experiment A. All tumors were treated with fractional irradiation by X-ray. Three of these cases are shown as follows:

Case 1. This shown primary cure (Plate VIII). The number of viable cells at points c and d are both 0; moreover irradiated field does not show its recurrence several years after treatment in clinical observation. In such cured cases, the period, from the end of treatment to the beginning of recurrence, is, of course, infinite.

Case 2. In Plate IX, a curve ascends again after treatment, and it indicates tumor recurrence. In figure c, of this Plate we can see several viable cells. The period, from the end of treatment to the point c, is 3 days.

Case 3. This also indicates tumor recurrence after treatment. In Plate X, at the lowest point b, the number of viable cells does not reach 0 and the curve ascends rapidly again to point c. In figure c of this plate we can see many viable tumor cells with mitotic figures. In this case, the period, from the end of treatment to point c, is 2 days.

Table 1

	within 1 week	〃 2 weeks	〃 3 weeks	〃 4 weeks	total
Number of cases	9	10	0	0	19
Percentage	47%	53%	0%	0%	100%

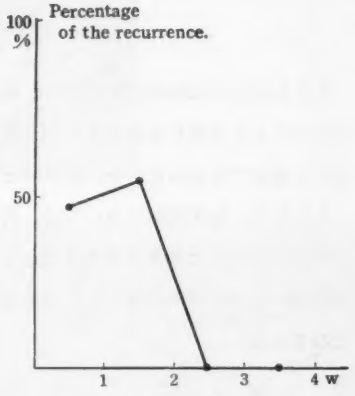

Figure 1

Result of experiment. As we have mentioned above, the latent periods, from the end of treatment to the beginning of tumor recurrence, were counted in all cases. The percentages of latent periods every week are shown in Table 1 and in Figure 1.

Conclusion

From the result of Experiment B, we can conclude that microscopical tumor recurrence, after fractional irradiation by X-ray, occurs within 2.5 weeks. In other words, after such treatment, we should look out for microscopically tumor recurrence for a period of at least 3 weeks. This result agrees with the result of animal experiment.

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要　旨

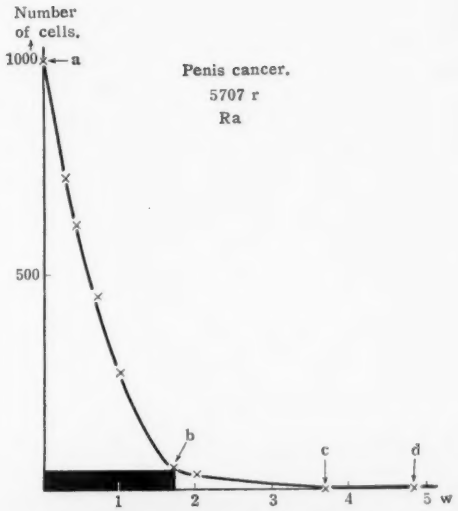
放射線による腫瘍組織の組織細胞学的変化　2. 臨床実験

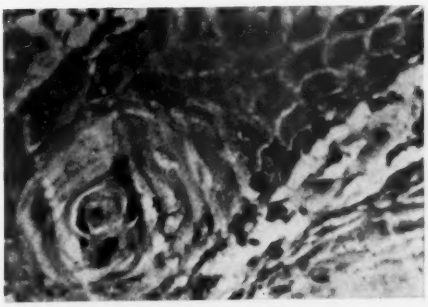
塚本憲甫，北川俊夫

(癌研究所附属病院放射線科)

先にわれわれは動物実験により，吉田肉腫のラジゥム照射後の致死的効果が充分に現われるには少くとも3週日を要するという結果を得たが，臨床上，しかもX線の分割照射を行った場合にも適応できるか否かを，前実験と同様の方法で皮膚癌 19 例に対して行った。

その結果，臨床実験においては，再発は，その照射方法の如何にかかわらず，組織細胞学的にすべて照射後より 2.5 週後まで起っており，これにより人癌（扁平上皮癌）の放射線治療後の腫瘍の致死的効果は少くとも 2.5 週日経過した後に始めて論ぜられるべきであることを知ったのである。





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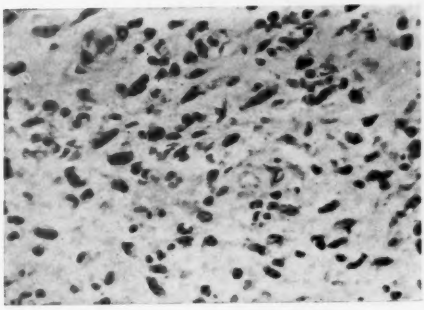

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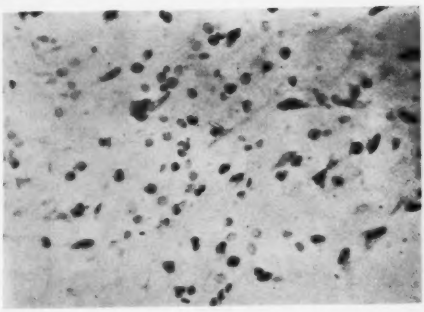

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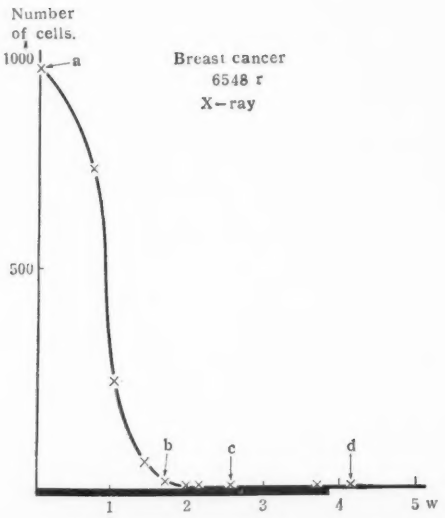
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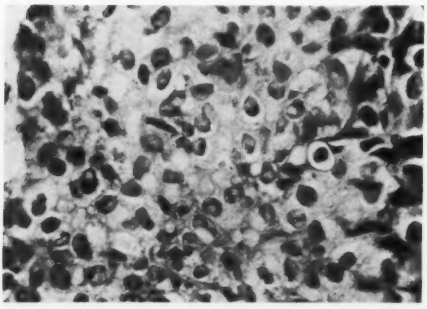

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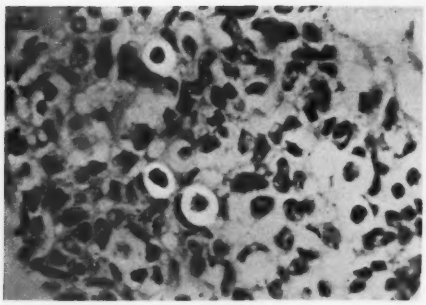

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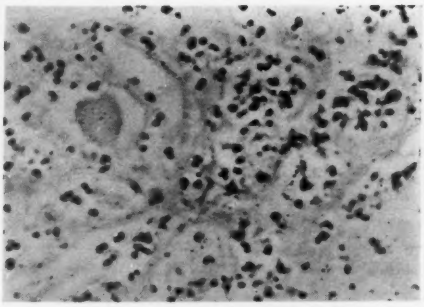

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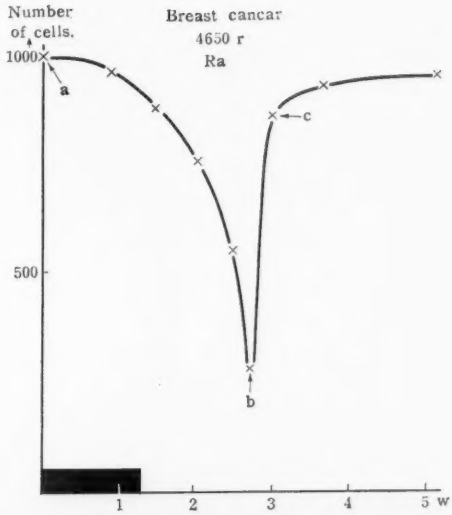

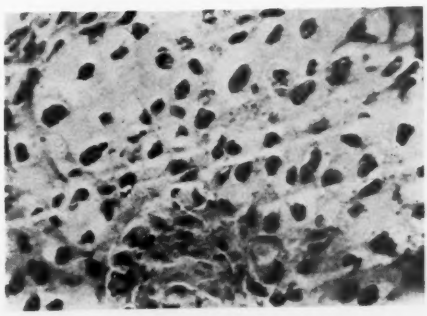

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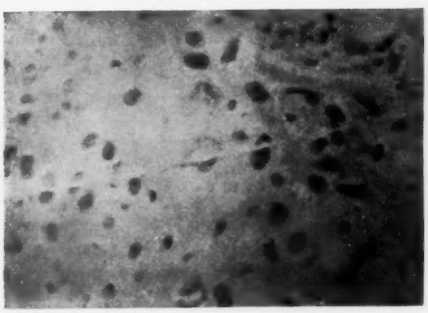

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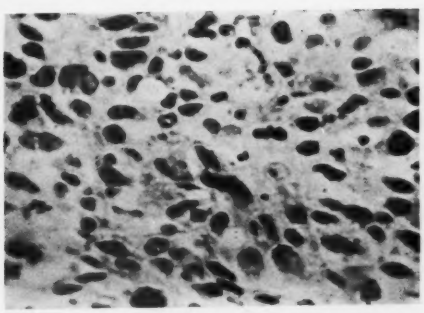

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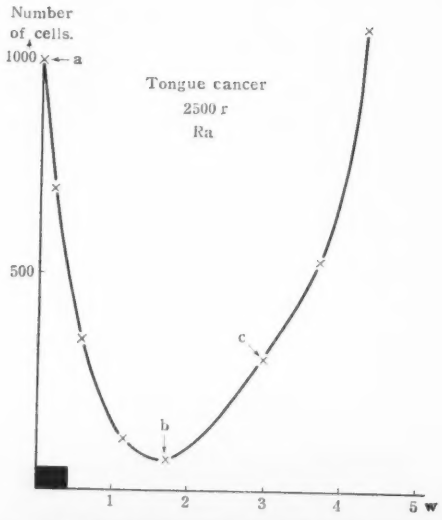

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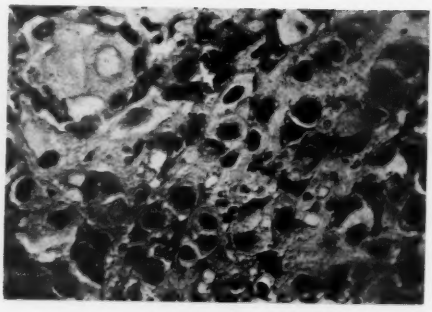

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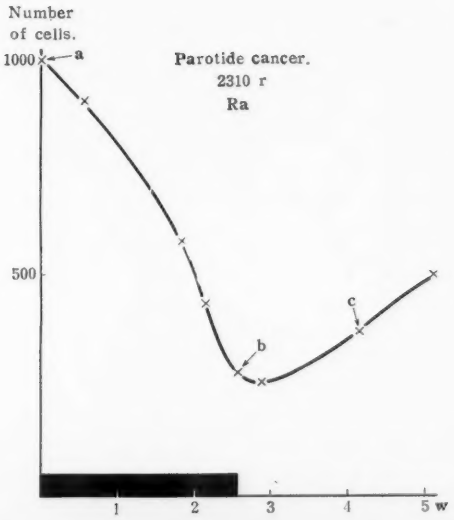
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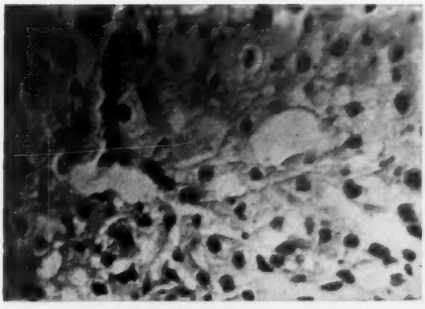

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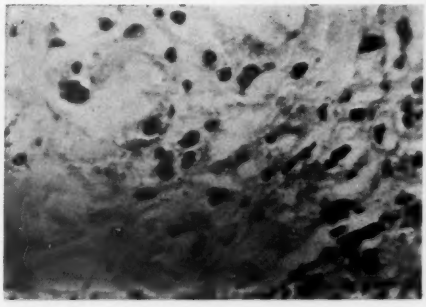

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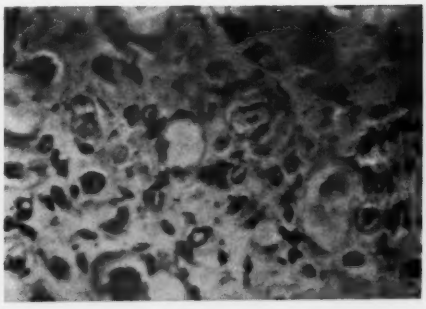

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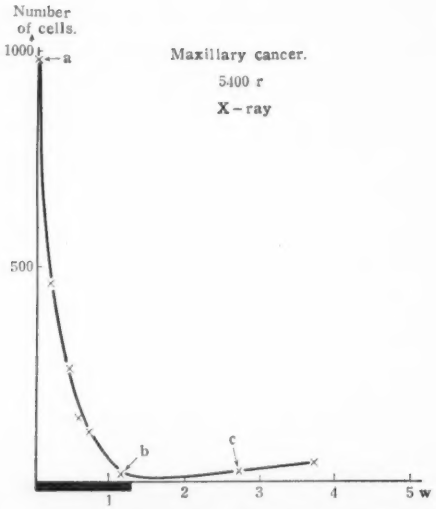
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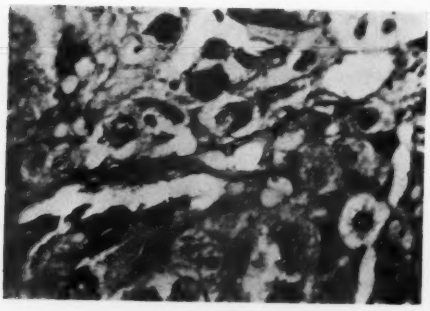

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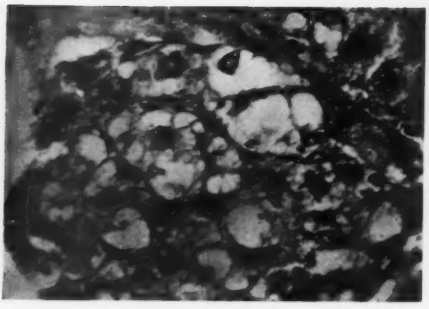

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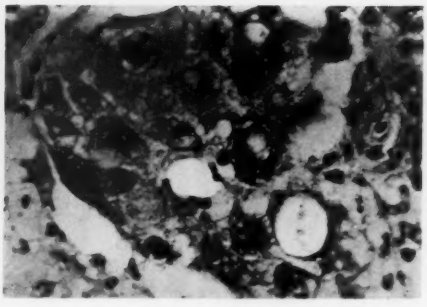

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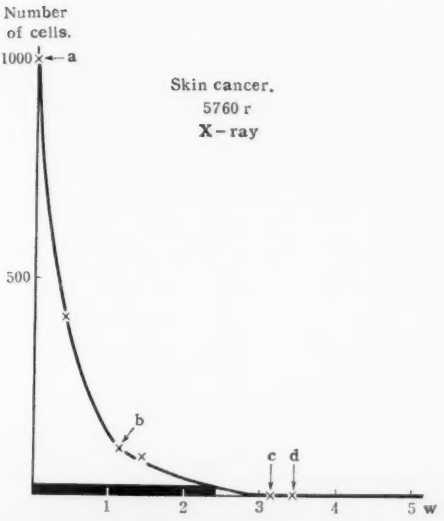

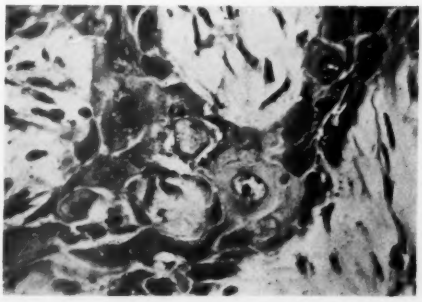

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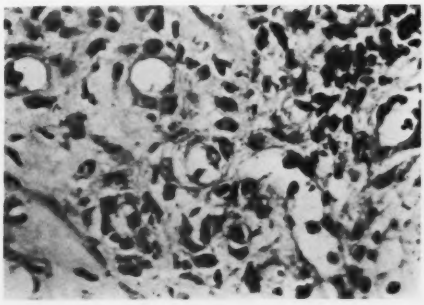

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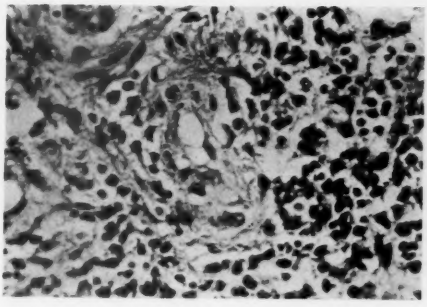

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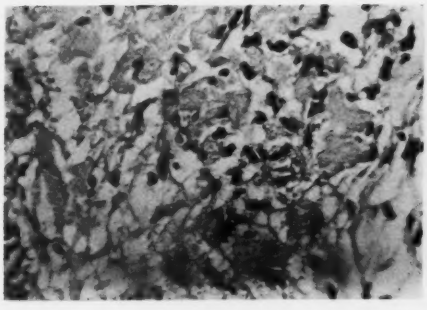

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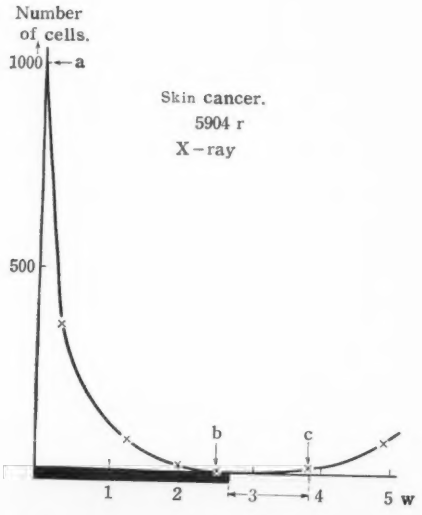

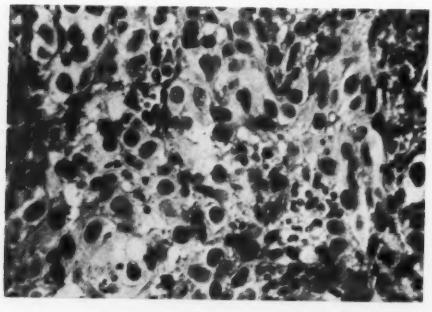

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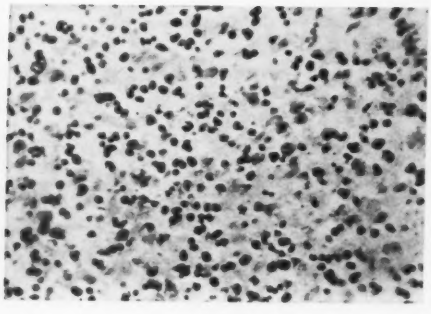

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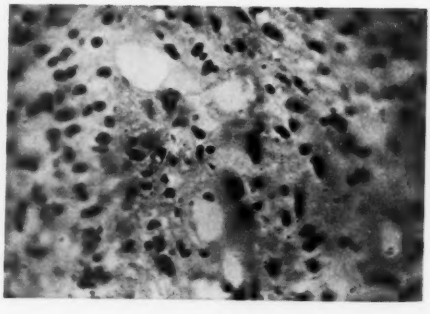

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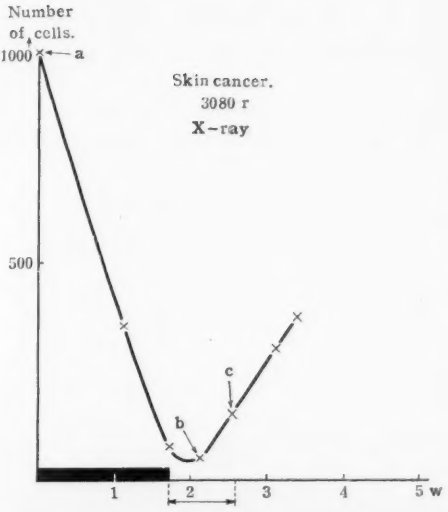

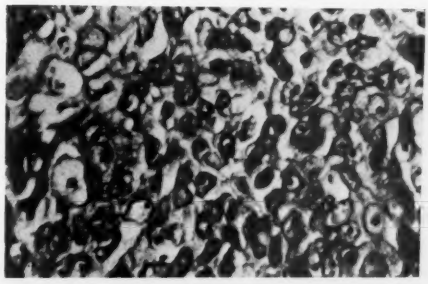

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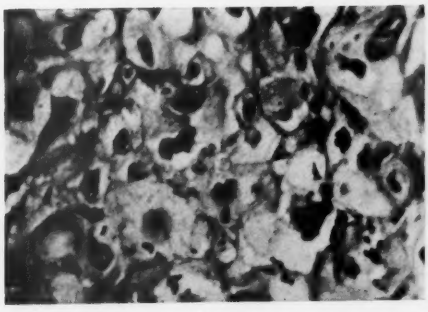

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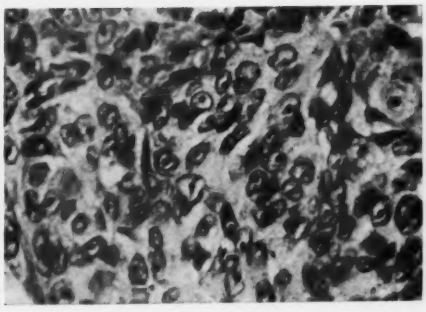

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[GANN, 49, 9~14; March, 1958]

ON A METHOD OF X-RAY TREATMENT FOR CARCINOMA METASTASIS IN CERVICAL LYMPHNODE

TOSHIO KITAGAWA
(Division of Radiology, Chief: KEMPO TSUKAMOTO, Hospital of Cancer Institute)

INTRODUCTION

Recently, radiation treatment of cancer, particulary for primary tumor, has made great progress, but for the node metastasis, a definite progress has not been recognized and only large field irradiation has been used generally as palliative treatment. Consequently, the surrounding tissue suffer severely from radiation injury and tumor can not be given suitable tumor dose and tumor grows up again soon after irradiation.

Therefore, in order to treat node metastasis thoroughly by radiation only, following conditions of irradiation are necessary:

1. Tumor should be given suitable dose by external irradiation.
2. Surrounding tissue of tumor should have the least injury after irradiation.
3. Minimum tumor lethal dose and its condition of irradiation should be determined.
4. Danger of latent metastasis which cannot be recognized at the time of irradiation should be known.

In this paper, author used the small field irradion by X-ray and reinvestigated every point I have mentioned above, and made sure of the possibility and indication of its treatment by X-ray.

EXPERIMENT

A) Irradiation

1) Cervical lymphnode metastasis of squamous cell carcinoma in head and neck region was irradiated by X-ray with small field. In this method, only the skin area under which lymphnode metastasis exists was irradiated, by covering the surrounding area with lead plate 2 mm in thickness. By lead protection, reaction of the skin by radiation was limited to a small area, and did not produce ulcer. Then we could not give the average dose of 7080r to the skin, whereas the average dose was 3500r by large field irradiation. This is shown in Table 1.

Accordingly, we can give a great amount of radiation dose to lymphnode metastasis under the skin, because injury of the skin is slight.

Table 1.

Case-number	Tumor dose(r)	Irradiated field (cm. ϕ)	Skin dose (r)
No. 1	5670	3	6554
2	4938	2	6563
3	5236	3	5424
4	6250	3	7053
5	6275	2	6563
6	8000	3	8475
7	6250	3	7052
8	5174	3	7345
9	7185	3	7618
10	5280	3	7080
11	5016	1	6270
12	3072	3	3704
13	6750	3	8475
14	6480	3	8136
15	6480	2	8400
16	6216	1	8568
		Average	7080

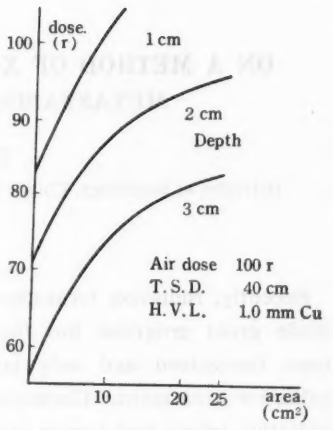

Fig. 1

2) The condition of irradiation. Tube voltage ; 180 kv, H.V.L. ; 1.0 mmCu, Filter ; 0.7 mmCu, 0.5 mmAl, Dose rate ; 53.5r/min, F.S.D. ; 40 cm, Daily dose (in air) ; 200–300r.

3) Tumor dose was caluculated with Quimby's table (1954). This is shown in Figure 1.

B) The determination of suitable period for extrcting irradiated lymphnode metastasis. In author's previous paper, it was clarified that tumor recurrence occurred within 3 weeks after irradiation.

Then, the possibility of tumor recurrence after irradiation should be judged after 3 weeks at least. From this reason, lymphnode was extracted 3 weeks after irradiation.

C) Each extracted lymphnode was made into histological section and it was examinated by the microscope to determine whether the viable tumor cell existed or not in its tissue.

If a viable tumor cell is not recognized in irradiated node, the node is judged as cured case. However, it was necessary to confirm whether metastasis had existed or not in node before irradiation, which was judged as cured case. The confirmation was achieved by examining following figures :

a) A trace of cancer island.

b) Carcinoma cell which shows karyopiknosis or karyorrhexis.

c) Remaining of horny layer which is special feature of squamous cell carcinoma.

d) Scar atrophy in part of nekrosis and its hyaline degeneration.

e) Definite white boundary between normal lymph tissue and trace of metastasis in macroscopic observation.

f) Definite yellow boundary between normal lymph tissue and nekrosis for cancer in macroscopic observation.

D) The determination of tumor lethal dose. 56 cases of lymphnode metastasis were examined after irradiation, as I have mentioned above.

All nodes were given the radiation dose in the range from 0 to 8000 r and divided into 4 groups by every 2000 roentgens, as shown in Table 2.

Table 2

Case-number	Tumor dose(r)	Size of the node (cm. φ)	Over-all time	Cure – Rec +	Cure -rate	Case-number	Tumor dose(r)	Size of the node (cm. φ)	Over-all time	Cure – Rec +	Cure -rate
1	1700	1.5	44days	+		29	3072	3.0	25days	+	
2	1980	3.0	21 〃	+		30	4280	3.0	36 〃	–	
3	1160	3.0	21 〃	+		31	4300	3.0	114 〃	+	
4	1160	3.0	21 〃	+		32	5500	3.0	39 〃	+	
5	492	3.0	4 〃	+	0%	33	5670	3.0	20 〃	–	
6	1020	3.0	6 〃	+		34	5360	3.0	24 〃	–	
7	472	3.0	5 〃	+		35	4222	3.0	24 〃	–	
8	525	3.0	8 〃	+		36	4050	3.0	21 〃	+	
9	746	3.0	9 〃	+		37	4938	2.0	29 〃	–	
10	1710	3.0	10 〃	+		38	5236	3.0	24 〃	–	68%
11	2160	1.5	43 〃	+		39	5445	3.0	35 〃	–	
12	2460	3.0	29 〃	–		40	5500	3.0	23 〃	+	
13	3480	3.0	40 〃	+		41	5174	3.0	34 〃	–	
14	2900	3.0	56 〃	+		42	4824	3.0	26 〃	–	
15	2320	3.0	21 〃	+		43	5280	3.0	18 〃	–	
16	2624	1.5	44 〃	+		44	5016	1.0	27 〃	–	
17	3198	3.0	58 〃	+		45	4508	3.0	46 〃	+	
18	2552	3.0	23 〃	–		46	6250	3.0	29 〃	–	
19	2904	3.0	49 〃	+		47	6275	2.0	31 〃	+	
20	3690	3.0	39 〃	+	26%	48	8000	3.0	38 〃	–	
21	3690	3.0	39 〃	+		49	6250	3.0	31 〃	–	
22	3986	3.0	42 〃	+		50	6216	1.0	25 〃	–	
23	3456	3.0	20 〃	+		51	6734	3.0	30 〃	–	90.9%
24	3964	3.0	43 〃	–		52	6750	3.0	30 〃	–	
25	2920	3.0	23 〃	–		53	6480	3.0	21 〃	–	
26	2660	3.0	21 〃	+		54	6480	2.0	23 〃	–	
27	2420	3.0	21 〃	+		55	7185	3.0	49 〃	–	
28	3625	1.5	36 〃	–		56	6875	1.5	32 〃	–	

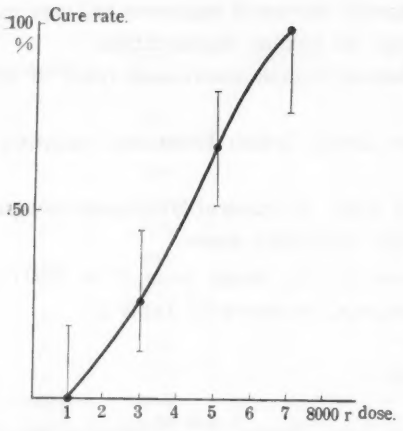

Fig. 2

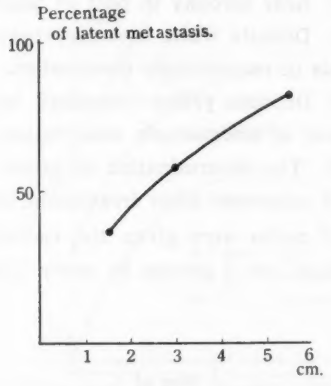

Fig. 3

Cure rate of each group shows the curve as shown in Figure 2. The tumor dose at 7000 r shows nearly 100% of cure rate. This dose is given from 3 to 5 weeks. Then, 7000 r of tumor dose should be determined as optimum lethal dose.

E) Indication of this method. When lymphnode metastasis is treated by only external radiation, there is a danger of missing other metastases existence so small that it can hardly be felt. If there are many of these latent metastases, this method of small field irradiation will become meaningless. Accordingly, it is necessary to study the relationship between node size and existence of latent metastases.

For this reason 100 cases of lymphnode metastasis of cancer in head and neck region were operated, with the result shown in Table 3. According to the node size, percentage of existence of latent metastases form curves as shown in Figure 3.

The danger-rate of lymphnode metastasis, more than 3 cm in diameter, is more than 50%. Consequently, in the case of single lymphnode metastasis less than 3 cm in diameter, this method is applied.

Conclusion

By this study, it is clarified that lymphnode metastasis can be cured by external radiation only. The treatment is achieved by X-ray with small field irradiation and 7000 r should be given to the metastasis from 3 to 5 weeks, without heavy injury to the surrounding tissue. Moreover, the indication of this method is the case of node size less than 3 cm in diameter.

Discussion

1) In this study, the high dose effect was not recognized, owing to the cover

Table 3

A	B	C	D	E	A	B	C	D	E
1	L	3.0	+	−	51	Tg	3.0	−	−
2	Tg	1.5	−	−	52	Tg	1.0	−	−
3	L	1.5	−	−	53	L	5.0	−	−
4	G	4.0	+	−	54	Tg	1.0	+	+
5	Tg	3.0	−	−	55	L	5.0	+	−
6	L	3.0	+	−	56	L	5.0	+	+
7	M	6.0	+	−	57	L	5.0	−	+
8	L	3.0	−	−	58	M	3.0	+	+
9	L	4.0	+	+	59	C	5.0	−	+
10	G	6.0	+	−	60	Tg	3.5	−	−
11	L	1.5	−	−	61	L	1.0	−	−
12	L	3.5	−	−	62	L	1.5	+	−
13	Tg	1.5	−	−	63	Tg	4.0	−	+
14	Tg	1.5	−	−	64	Tg	2.0	+	+
15	Tg	1.5	−	−	65	Tg	1.5	−	−
16	M	3.0	+	−	66	Tg	2.0	−	−
17	L	5.0	+	+	67	Tg	4.0	−	−
18	L	4.0	−	−	68	Tg	5.0	+	+
19	L	1.0	−	−	69	L	3.0	−	−
20	L	1.0	−	−	70	P	1.5	+	+
21	Tg	3.0	−	−	71	L	5.0	+	+
22	P	5.0	+	+	72	M	3.0	+	−
23	Tg	3.0	−	+	73	L	3.5	−	−
24	Ts	3.0	−	+	74	Tg	3.0	−	−
25	L	1.0	−	−	75	L	5.0	+	+
26	Tg	4.0	−	−	76	L	1.5	−	+
27	Ts	4.0	−	−	77	M	3.0	−	−
28	Tg	1.5	−	−	78	L	3.5	−	−
29	M	1.0	−	−	79	L	5.0	−	−
30	L	4.0	+	−	80	C	1.0	+	−
31	L	1.0	−	−	81	Tg	1.5	+	+
32	Ts	4.0	−	−	82	M	3.0	−	−
33	Tg	6.0	+	+	83	M	1.5	−	−
34	L	1.5	−	+	84	G	3.0	+	−
35	L	1.5	−	−	85	L	3.5	−	+
36	Tg	3.0	+	−	86	L	1.5	+	−
37	Tg	2.0	−	+	87	L	4.0	+	−
38	Tg	1.5	−	−	88	L	4.0	+	−
39	L	4.0	−	−	89	Tg	3.0	+	−
40	C	3.0	−	−	90	Tg	1.5	+	−
41	Tg	3.0	+	−	91	Tg	3.0	+	−
42	L	5.0	+	+	92	L	3.5	+	+
43	M	1.0	−	−	93	Tg	3.0	+	+
44	L	5.0	−	−	94	L	1.5	+	+
45	Tg	3.0	+	−	95	Tg	3.5	−	−
46	L	3.5	−	+	96	L	4.0	−	−
47	M	3.5	+	−	97	L	2.0	−	−
48	L	1.5	+	−	98	L	1.5	−	−
49	L	3.0	−	−	99	L	3.5	−	−
50	L	1.5	−	−	100	L	5.0	+	+

A ; Case-number B ; Region of the primary tumor
C ; Diameter of the lymphnode metastasis (cm)
D ; Latent metastasis : positive (+), negative (−)
E ; Recurrence after 6 months from operation positive (+), negative (−)
Larynx ; L, Pharnx ; P, Tongue ; Tg, Gingiva ; G
Tonsil ; Ts, Cheek ; C, Maxillary sinus ; M

we used over the surrounding tissue.

2) Hitherto, it has been said that the optimum lethal dose for squamous cell carcinoma is 6000 r. But in this study, the optimum dose for its lymphnode metastasis was found to be 7000 r.

3) Differynce of lethal dose in consequence of the difference of tumor size was not studied because it is meaningless in practical use for the tumor more than 3 cm in diameter.

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要　旨

リンパ節転移癌の一治療法について

北　川　俊　夫（癌研究所附属病院放射線科）

リンパ節転移癌の治療は従来ほとんど手術的に行われ，放射線による治療は著明な進歩を示していない。著者は鉛板で周囲の皮膚，軟部組織を蔽い，これらを放射線より防護することにより，すなわち小照射野法を用いて目的を達したが，この方法を用いた場合，与えるべき線量およびその適応についてさらに研究を行った。

すなわち 3～5 週日内に平均 7000 r を与えるのが最適であり，転移リンパ節の長径が 3cm 以下のものにだけ，この治療法を適応する意義があることを知った。

[GANN, 49, 15~21; March, 1958]

CARCINOMA OF THE SKIN AND ITS RADIATION TREATMENT

KEMPO TSUKAMOTO and TOSHIO KITAGAWA
(Division of Radiology, Chief: KEMPO TSUKAMOTO, Hospital of Cancer Institute)

Introduction

The radiation treatment of skin cancer is one of the most interesting problems in radiotherapy for the varities of treatment methods. Carcinoma of the skin originates on the surface of the body, so we can diagnose it easily and we can select the most suitable method of treatment for it. Although various methods of radiotherapy have been carried out, there seems to be a certain rule for selecting the method.

We have investigated 155 cases of skin cancer which we treated during 1946 to 1955, and analysed the result of our radiation treatment as well as other interesting problems concerning it.

Incidence

The average age of the onset of skin cancer is about 65 years, although it is not rare also in younger patients, as the following figure (Fig. 1) shows.

In our cases, there are two patients who are under ten years old, and in both of them cancer occurred on the base of Xeroderma Pigmentosum. Men are affected about three times as often as women.

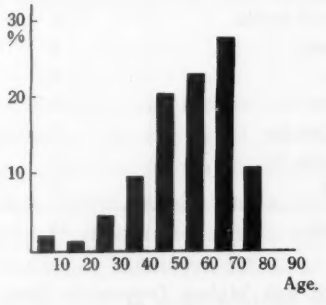

Fig. 1

Table 1

	Number of cases
Burn	16
Wound	16
Verruca	15
Chronic eczematoid dermatitis	9
X-ray irradiation	5
Xeroderma pigmentosum	2
Frostbite	1
Total	64

Aetiology

It is quite interesting that in many cases of skin cancer there are definite facts

about their causes.

In Table 1, the most frequent cause of the tumors is from the scar of the burns and wounds. In the cases of burns, most of them are caused by fire and very few of them caused by hot water. They usually occure as late as 30 years after the incidence.

There are a few but interesting cases of roentgen cancer of which we should discuss later. Most of them are caused by surface irradiation of low voltage X-ray, applied for a certain length of time, for the purpose of treating chronic skin diseases. Recently low voltage X-ray therapy for chronic dermatitis is not commonly used, and we hope such a kind of skin cancer will not appear anymore.

As we mentioned above, in many cases of skin cancer there exist definite causes. In cases of skin disease which have chronic course with chronic ulcer infiltration or induration, we should take the biopsy and examine it carefully.

The Difference in Frequency of Growth on Different Parts of the Body

Another interesting character of skin cancer is great difference in the frequency of growth in the parts of the body which is shown in Table 2.

In addition, Table 2 indicates that many cases of skin cancer arise from the parts of the body exposed to the air, for example the face or the arm. It is shown in Table 3.

Table 2

Face	32%
Head and Neck	22%
Extremity	30%
Trunk	9%
Other parts	7%

Table 3

	Number of cases
Forehead	3
Upper eyelid	3
Lower eyelid	11
Cheek	5
Ear	4
Nose	7
Upper lip	1
Lower lip	7
Jaw	3
Total	44

It is an interesting fact that the lower eyelid has higher frequency than the upper one and the same relation existed in regard to lips.

Histological Classification

We classified 88 cases, which we were able to examine their histological charac-

ters minutely. They are shown in the following table (Table 4).

Table 4

	treated cases	cured cases
Squamaus cell Ca.	59	24
Basal cell Ca.	11	8
Malignant Melanoma	11	4
Ca. simplex	1	0
Sweat gland Ca.	2	2
Syringadeno-ca.	1	1
Bowen's disease	2	2
Sarkom	3	0
Total	90	41

Squamous cell carcinoma is found more commonly than basal cell carcinoma in Japan compared to America and Europe. Usually the basal cell carcinoma does not produce any secondary metastasis in lymphnode and the primary tumor grows slowly. However we have also the experience of the case of the transitional form between squamous cell carcinoma and basal cell carcinoma, so we should keep it in mind that, even if it is basal cell carcinoma histologically, there is still a certain risk of producing metastasis. Moreover, there are two kinds of malignant melanoma, i.e. with or without melanin pigment, and they both are quite malignant in nature. Sometimes we have the experience in Japanese that a certain cases of basal cell carcinoma of the skin with melanin pigment. Therefore we should be carefull about the differential diagnosis between basal cell carcinoma and malignant melanoma. We have a few carcinoma simplex or sarcoma in our cases. They are worst in their prognosis.

From the above mentioned facts, we know that grade of malignancy in skin cancer is widely variable, so we should take the biopsy from the tumor before we treat it, because it is quite necessary for the determination of treatment policy. However, with malignant melanoma, we should not take the biopsy carelessly for this procedure has the risk of causing metastasis.

Treatment

1) Method. The method of radiotherapy of skin cancer given in literature is as follows: a) For small tumor, less than 2 cm in diameter, superficial growth, tumor dose, more than 6000r, should be given by β ray or low voltage X-ray. b) For larger tumor than a, radium needling or X-ray fractional irradiation or contact irradiation by Chaoul apparatus should be used. c) The radiation effect of malignant melanoma should not be expected highly, because of its less radiosensitivity. It is asserted that we would rather use operation for its treatment.

Our treatment policy is as follows: Most of our patients, when they first visit our hospital, usually have too large a tumor already; in other words, their size is more than 2 cm in diameter. Therefore we would rather use following two methods: One method is radium needling. If tumor dose is not sufficient by

needling alone, it is followed by X-ray irradiation. Another method is X-ray irradiation or external irradiation of radium followed by radon gold seed implantation.

In many cases of the tumor, more than 2 cm in diameter, the external radiation alone will leave some tumor nests after the treatment. Consequently we should not depend upon the treatment of external radiation only.

2) There are differences in the results of treatment of skin cancer by their clinical stages in each case. We divided all our cases as follows, according to our own definition, to each clinical stage before treatment:

Stage I; The lesion is limited to the surface, less than 5 cm in diameter. No definite metastasis is present.

Stage II; Size of the tumor is the same as stage I, or more than 5 cm in diameter. One metastasis is present, but mobile.

Stage III; Tumor spreads deeply and its area is more than 7 cm in diameter. Many fixed metastases are present.

Stage IV; With distant metastasis.

Figure 2 shows the relationship between survival rate in years after the treatment, according to the classification described above. In addition, the tendency of all cases in survival rate is shown in Figure 3. In Fig. 2, there is a remarkable difference in survival rate between stage I, without metastasis, and all other stages, with metastasis. It indicates that the treatment of skin cancer after the occurance of the metastasis is very difficult. The treatment of skin cancer, as a matter of fact, is not so simple although it is a superficial growth.

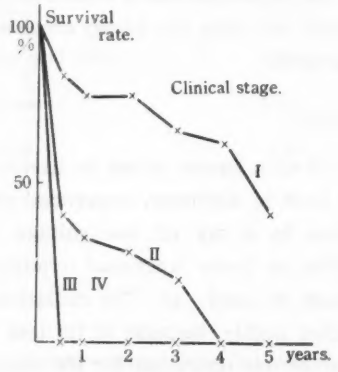

Fig. 2

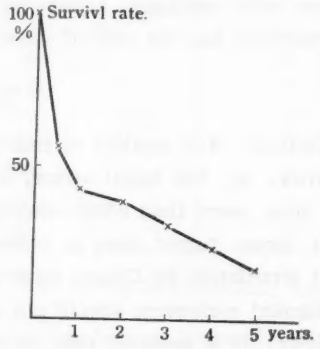

Fig. 3

3) The relationship between dose given and cure rate. In this experimental study, we used 38 cases of squamous cell carcinoma of skin cancer, treated by γ ray emitted from radium. Tumor dose is caluculated by Paterson, Parker's dose-

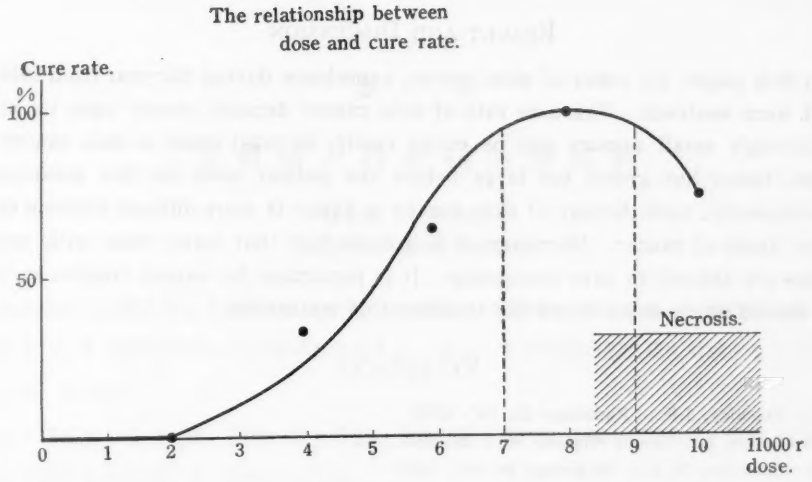

Fig. 4

table and we gain a curve as shown in Fig. 4. This curve begins to rise at the point of 2000r, after which cure rate increases gradually as the dose increases, and arrives at the maximum point at 8000r of tumor dose with 100% of cure rate. However, the more the dose increases, the cure rate decreases on the contrary. In addition, average 8000r of tumor dose is given to the tumor of 3–5 cm in diameter, during 8–11 days as the over-all time. And it is clear that high dose effect begins to appear at the point more than 8000r.

4) The problem between other histological character of skin cancer and therapeutic dose. a) We have attain good results for the cases of basal cell carcinoma by giving dose less than 6000r. b) We could not get good results for most cases of malignant melanoma, but in only few cases, we could cure it by giving the dose more than 9000 r.

We are sure that all cases of malignant melanoma are not always contraindication for radiotherapy, because few cases of them have certain radiosensitivity. Therefore, we should try radiotherapy on it with full dosage at a time, if the condition of sorrounding tissue is favorable for it.

5) Treatment of lymphnode metastasis. We used chirurgical treatment for the most of our cases. Radiationtherapy did not attain good results until today. Recently, however we are trying the method of small field irradiation by X-ray which is effective for some cases.

Radium needling and other methods are being tried in America and Europe, and we believe that radiationtherapy for lymphnode metastasis will advance.

Result and Discussion

In this paper, 155 cases of skin cancer, experience during the year from 1946 to 1956, were analysed. The cure rate of skin cancer depends chiefly upon its size.

Although small tumors can be cured easily, in most cases of skin cancer in Japan, tumor has grown too large before the patient asks for the treatment. Consequently, radiotherapy of skin cancer in Japan is more difficult problem than other kinds of cancer. Moreover, it is definite fact that many cases with metastases are difficult to cure completely. It is important for cancer treatment that we should study more about the treatment of metastasis.

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要　旨

皮膚癌の放射線治療成績

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われわれは 1946 年より 1955 年までの間に治療した皮膚癌の 155 例を，一般性状の特に興味ある 2，3 の事項について分析するとともに，主として放射線治療成績を対象として検討を行った。すなわち

1. 誘因としては火傷，創傷が最も多く，身体の露出部に発生率が大であり，眼瞼，口唇にては下部のものに特に多く発生している。

2. 組織学的には，欧米では基底細胞癌が最も多いのに対し，扁平上皮癌が最も多く，また基底細胞癌が最も予後がよい。

3. ラジゥムを用いての治療の場合は 3~5 週日に，平均 8000 r を腫瘍に与えるのが最適と考えられる。

4. 皮膚癌は体表に存在するため治療が比較的容易である如く考えられるが，現在のわが国においては，欧米の報告にあるものよりも，初診時すでに臨床進展の進んだものが多いので，その治療はなお楽観を許さないものがある。

[GANN, 49, 23~26; March, 1958]

RADIATION TREATMENT OF CARCINOMA OF THE THYROID

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INTRODUCTION

Radiation treatment of thyroid carcinoma has not been regarded as of great importance because of its low radiosensitivity. Recently, however medical use of isotope has made great progress in the therapy and diagnosis of thyroid tumor, and we have taken this opportunity to reinvestigate our results of radiation treatment. In this report, we analysed 97 cases of thyroid carcinoma previously treatted by radiation during 1946-1955.

Grading of Clinical Stage and its Survival Rate.

We classified our cases by Jacobsson's grading of clinical stage (1954), and found that each group representing one of the following four grades, divided in 4 groups shows its own characteristic survival curve, as indicated in Figure 1:

Grade I: Primary tumor is mobile and has no metastasis.

Grade II: Primary tumor is mobile or slightly fixed and only one mobile metastasis is present.

Grade III: Primary tumor enlarged and invaded the sorrounding tissue and heavily fixed. Many fixed metastases are present.

Grade IV: With remote metastases.

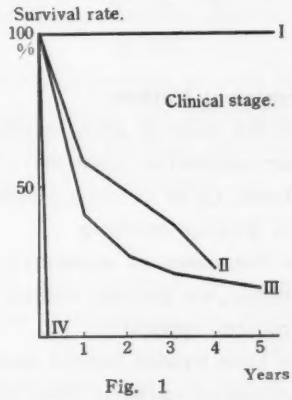

Fig. 1

Radiosensitivity

Many authors have described the low radiosensitivity of thyroid carcinoma. We also have experiences that many thyroid tumors show slight diminution in their sizes long time after the irradiation or show no change at all.

In our cytological experimental work, we compared radiosensitivity of thyroid carcinoma with breast carcinoma, by counting numbers of viable tumor cells in certain microscopic area, after X-ray irradiation under the same conditions. Changes in number of viable cells in the two kinds of cancer according to time after irradiation are shown in Figure 2, which indicates that the radiosensitivity of thyroid cancer is much lower than that of breast cancer, in spite of the fact that they are both adenocarcinoma and have the same kind of histological picture.

Histological Classification

We classified 49 cases of thyroid carcinoma in which their histological pictures are clear, by S. Warren and W.A. Meissner's classification. They are shown in the following table (Table 1). From this table, it is clearly recognized that squamous cell carcinoma, anaplastic carcinoma and spindle cell carcinoma have worse prognosis than adenocarcinoma in speite of fheir relatively high radiosensitivity.

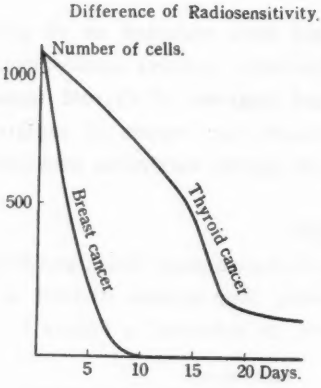

Fig. 2

Table 1

Adenocarcinom	case	metas-tasis	dead cases
Papillary type	31	16	12
Cystpapillary type	7	3	2
Follicular type	4	3	0
Squamous cell carcinoma	3	2	2
Anaplastic carcinom	2	2	2
Spindle cell tumor	2	1	2
total	49	27	20

Treatment Method

For the cases of adenocarcinoma we apply, 1) Operation added by external radium application (2500-3800 r) or teleradium irradiation. 2) External irradiation by X-ray, Co 60 or radium (4000-4500 r) added by gold Rn-seed implantation, and / or 3) Radium needling.

To the cases of squamous cell carcinoma or anaplastic carcinoma or spindle cell tumor, we practice radium needling or Rn-seed implantation added by external radium application.

We have treated thyroid carcinoma usually by the above mentioned plan. External use of radiation alone does not usually show sufficient effect for thyroid carcinoma except in the cases of early stage, because of the low radiosensitivity. Therefore, we irradiated it by X-ray, Co 60 or teleradium (4000-5000r), and after that added Rn-seed implantation.

As for radium needling, its end results are often unfavourable, for instance, it has the danger to induce distant metastasis. So we rather prefer Rn-seed implantation to radium needling from the viewpoint of safety.

Result and Discussion

The relationship of survival rate in both cases treated by radiation alone or

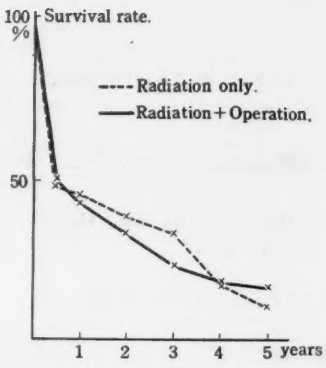

Fig. 3

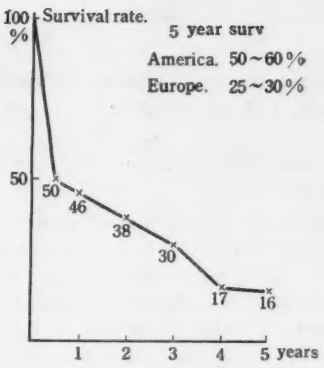

Fig. 4

treated by operation with radiation are shown in Figure 3. Although the survival rate is 100% for the cases in early stage treated by operation with radiation, as a whole, including both II and III grades, the curve of survival rate in the cases treated by operation with radiation shows the descending tendency. So we cannot find the distinct difference, on the whole, between these two kinds of treatment described above. From our results, it seems better to use only radiation for the cases of II or III grades. The survival rates of all cases which we have treated are shown in Figure 4.

Radiosensitivity of the thyroid tumors differs greatly according to their histology. Therefore it is very important to take biopsy before the treatment, in order to decide whether the case should be treated by radiation alone or by operation. The prognosis will be greatly influenced by the treatment.

Summary

We analysed 97 cases of thyroid carcinoma which were treated by radiation during 1946-1955 and tried to reinvestigate it on the standpoint of the following facts:

A) There exists a close relationship between histological figure of tumor and its prognosis, and we must use different treatments for different types of tumor. It is imperative that we take biopsy before the treatment, in order to determine the most suitable form of treatment.

B) The tumor which has higher radiosensitivity is rather worse in its prognosis than the one with lower sensitivity.

C) Although the cases in the first stage are fit for the treatment by operation with radiation, we would rather use the treatment by radiation alone for the cases in II or III stages.

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要　旨

甲状線癌の放射線治療

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われわれは 1946 年より 1955 年までの間に治療した 97 例の甲状腺癌症例を，主として放射線治療の見地から分析考察して次の結果を得た。

1. 5 年生存率は 16%であった。

2. 放射線感受性の高い腫瘍は，その低いものより予後が悪い。

3. 臨床進度Ⅰ度のもに対しては，手術に放射線照射を併用して 100%の治癒率を得ているが，Ⅱ，Ⅲ度の症例においては放射線だけを用いて治療したものと，手術との併用治療したものとの治療成績に明らかな差異を認めないので，現在われわれはⅡ，Ⅲ度の症例には放射線のみによる治療を行っている。

4. 治療を始める前に組織学的性状を確めることは，診断の確認，放射線感受性の推測，予後の推定上特に重要である。

[GANN, 49, 27~31; March, 1958]

PRODUCTION OF RAT SARCOMA BY INJECTIONS OF TWEEN 80 SOLUTION OF SCARLET RED

(Plates XI and XII)

MASAO UMEDA

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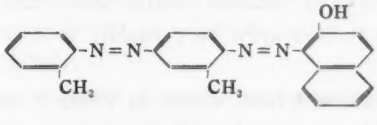
The earliest work on the cell proliferative properties of the azo dyes dates back to 1906 when B. Fischer (1) injected solutions of scarlet red into the ears of rabbits. The resemblance of the cell proliferation induced by scarlet red to a cancerous growth impressed Fischer and many other authors (Jores (2), Stahr (3), Helmholz (4), Seckel (5), Benthin (6), Haga (7), Yamagiwa (8), Yoshida (9), Kinoshita (10), and Shear (11)) who subsequently repeated, confirmed and extended Fischer's original work on scarlet red, but all of these experiments failed to produce subcutaneous sarcoma.

Recently I carried out an experiment with scarlet red, using tween 80 as solvent, with the surprising result that sarcoma developed in 50% of the rats.

Experimental Methods

The sample of scarlet red used was obtained from the Grübler Co., Ltd., and tween 80 was a product of the Atlas Powder Co., U. S. A.



The animals used were albino rats of the Saitama strain, all weighing around 200 g. They were kept in wire cages in groups of five each, and were maintained on the usual laboratory diet of whole wheat with occasional supply of dried fish and green vegetables.

Scarlet red was dissolved in tween 80 at the concentration of 2 gdl, and was injected subcutaneously on the back of the rats in 0.2 cc amounts. Injections were repeated once every week, and scarlet red solution was sterilized by heating before injection.

Results

Of the 24 rats, with which the experiment was started, 8 rats survived the injection period of 403 days, receiving 32 injections during the period. Of 8 rats which survived for more than 403 days, 4 developed sarcomas at the site of injection at 403, 427, 428 and 463 days respectively (Table 1).

Table 1. Experimental Data on 8 Rats Surviving over 400 Days.

Rat No.	Sex	Exp. days	Total amount injected: Scarlet red (mg)	Total amount injected: Tween 80 (cc)	Inject. No.	Body Weight (Final) (g)	Tumor size (cm)	Histological diagnosis
5	m	504	188	9.4	44	230		
8	f	438	140	7.0	35	177	4.6×3.6×2.9	Fibrosarcoma
9	m	424	140	7.0	35	140		
10	f	445	144	7.2	36	190		
15	m	485	176	8.8	42	223	3.9×3.2×2.5	Fibrosarcoma
22	m	407	128	6.4	32	290	3.0×4.2×2.0	Rhbdosarcoma
23	m	497	176	8.8	42	230	5.5×4.3×2.5	Fibrosarcoma
24	m	498	176	8.8	42	168		

Three rats died within 200 days after the beginning of the injections, and these rats all showed severe pneumonia.

The subcutaneous connective tissue of the rats survived 200 days or more became thickened and small, hard consolidations containing the dye, with ulceration, became palpable during the 7th to 8th months. These consolidations turned into irregular nodules with red pigment persisting for a long time. In this period, the consolidation developed rapidly into tumors in 4 rats. As the tumors increased in size they became clearly demarkate from the surrounding connective tissue. The tumors grew very rapidly to a very large size, and soon killed the animals.

Individual records of these 4 rats, shown in Table 1, are as follows:

No. 22. A hard nodule of the size of thumb-tip was palpated on the 403rd day. The animal was killed on the 407th day with the tumor measuring 3.0×4.2×2.0 cm surrounding ulceration.

No. 8. A nodele measuring 3.1×2.7×1.9 cm was palpated on the 428th day. The animal was killed in very weakened condition on the 438th day with the tumor measuring 4.6×3.6×2.9 cm.

No. 15. The tumor was found as a hard nodule of the size of middle finger-tip on the 463rd day. On the 477th day it reached the size of 3.7×3.1×2.6 cm, which grew to the size of 3.9×3.2×2.5 cm by the 485th day, when the animal was killed.

No. 23. A hard nodule of the size of the thumb-tip, first palpated on the 427 th day, grew to the size of 4.3×3.1×2.2 cm on the 441 st day. The animal died on the 492 nd day with the tumor measuring 5.5×4.3×2.5 cm.

Findings at Autopsy. On cutting open the tumors, a considerable amount of the solution of scarlet red was seen in the center of each. They were mostly elastic hard but some of them were softer. The cut surface was generally pearly white, occasionally with pinkish or redish areas due to hemorrhage, with hardly recognizable necrotic areas. These tumors were thinly encapsulated, clearly defined from the surrounding tissue to which they were slightly adherent. Of special interest in all these tumors was the fact that scarlet red solution remained in the center (Plate XI, Fig. 2).

No macroscopic metastasis was found in any of the cases.

In the course of the experiment, it was noted that the local tissue, in which there was no sarcoma production, showed chiefly fat granuloma with necrosis keeping red color due to the absorption of the pigment. The liver was congested and an increase of Kupffer's stellate cells and vacuolar degeneration of liver cells were observed. Spleen showed fibrosis. Lymphnodes showed hypertrophy of reticulum cells and increase of lymphocytes.

Histology of the Tumors. All tumors were composed for the most part of irregularly interlacing bundles of long spindle shaped cells of the fibrosarcoma type. In one of the tumors was evidence of the active invasion of the tumor tissue into the muscular layer (Plate XII, Fig. 5). Mitoses were numerous in most tumors, and giant cells and polymorphic cells were found only in limited areas.

Transplantation. Attempts to transplant the tumor to normal rats were made in the case of Rat No. 8. The transplantation from this tumor took in 43% of the animals in the first generation, 90% in the second, 100% in the third, 80% in the fifth, etc. The transplanted rats died of tumor in about one month on an average after transplantation.

Microscopically, the transplanted tumor was classed as fibrosarcoma identical with the original tumor.

Discussion

The first question that arises in the above experiment is whether tween 80 is in itself carcinogenic, as in the case of my previous experiments with m-toluylenediamine (12). But in the experiment of propylene glycol solution of p-quinone (1957 (13)), it was proved that propylene glycol itself has no carcinogenic activity. However, I think that the solvent is a factor which is able to enhance the effect of carcinogen, that is, to act as cocarcinogenic agent in the cellular environment. Setälä, et al. (14) reported that the most potent as solvents are

to be found among non-ionic compounds which contain long-chain fatty acid in their molecule and which at the same time are comparatively watersoluble. He pointed out also that especially certain Tween-type materials belong to this category. Riska (15) reported tumor induction using Tween-type solvents. In my opinion it seems that tween 80 is a kind of Tween-type materials which acts as a promoting factor. The effect of the solvent on experimental subcutaneous carcinogenesis is a problem much too complex to be exhaustively discussed at the present time.

In future I shall consider the question of the solvent effect in all its aspects.

Summary

Sarcoma was produced at the site by repeatedly injecting 2 gdl solution in tween 80 of scarlet red subcutaneously into normal rats. Injections were made in doses of 0.2 cc at as nearly the same site as possible on the back, and at the rate of once a week for about 13 months, and sarcoma developed in 4 of 8 rats that survived this period. In these tumors, a considerable amount of the scarlet red was seen in the center.

I have to acknowledge my deep gratitude to Dr. W. Nakahara and Dr. M. Tanaka for cordial instruction in the preparation of this paper.

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Explanation of Figures (Plates XI and XII)

Fig. 1. Fibrosarcoma arising from the administrations of scarlet red on the 438th day.

Fig. 2. The above tumor cut open, showing the presence of scarlet red solution held in the center of the tumor.

Fig. 3. Histological picture of the inner margin of the above tumor surrounding the solution of scarlei red. The dye had disappeared in the preparation of the tissue and left an empty space.

Fig. 4. A typical fibrosarcoma. Rat 8 (438 days)

Fig. 5. Invasive growth of sarcoma into the mascular tissue. Rat 22 (407 days).

Fig. 6. Histology of the tumor in rat 15 (485 days).

Fig. 7. Histology of the tumor in rat 23 (492 days).

要　旨

Scarlet red によるラッテ肉腫の発生

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(癌 研 究 所)

Scarlet red による発癌実験は 1906 年 Fischer がオリーブ油にとかして，ウサギの耳の皮下に注射して上皮の異型増殖をみたのがはじまりで，その後山極らが鶏輸卵管上皮性腫瘍，Schmidt, Korteweg らが肝腺腫，Maisin, Picard, Puccinelli らが乳嘴腫を作ったにとどまる。私は先の実験の m-toluylenediamine が溶媒を propylene glycol にすることによって，ラッテの皮下に 100%肉腫ができたことにかんがみ，最近 Truhaut らによって tumor promoter といわれている Tween を Scarlet red の溶媒として用いた結果，8匹のラッテのうち4匹に皮下肉腫を発生することができた。

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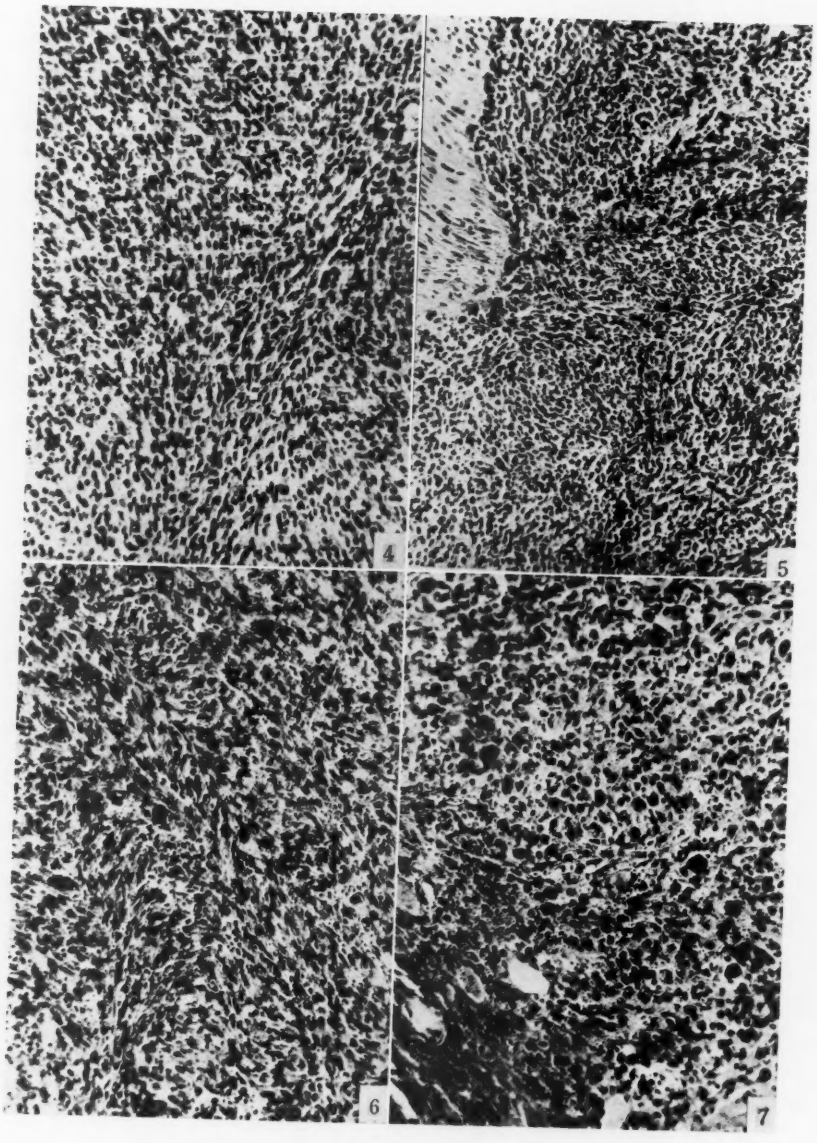
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[GANN, 49, 33~41; March, 1958]

THE RELATION BETWEEN CARCINOGENICITY AND CHEMICAL STRUCTURE OF CERTAIN QUINOLINE DERIVATIVES*

(Plate XIII)

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and SUMIO SAKAI
(Laboratories of the Kaken Chemicals, Ltd., Tokyo)

INTRODUCTION

A recent publication from our laboratories reported experiments which demonstrated that 4-nitroquinoline N-oxide is a powerful carcinogen (1). It was found that the substance in 0.25 percent solution in benzene, when painted on the skin of normal mice three times a week, produced papillomata in all the animals between 80th to 140th day, and malignant tumors between 120th to 200th day. The malignant nature of these tumors was established by their pathological characters (squamous cell carcinoma or sarcoma, with occasional metastasis), their biochemical properties (high anaerobic glycolysis), as well as by their transplantability. That 4-nitroquinoline N-oxide is a carcinogen of the potency approaching that of the most powerful polycyclic hydrocarbons cannot fail to be of special interest from a chemical point of view, not so much because the substance belongs to a hitherto unsuspected group of bicyclic compounds, but perhaps more on account of the relatively simple chemical structure, which makes it highly commendable for a study of the relation between the chemical structure and carcinogenicity.

As we pointed out in our previous paper, there are reasons to suspect that the nitro radical at the position 4 may be the part of this molecule actively concerned in the carcinogenic action. We recalled the statement of Ochiai (2), who is responsible for the first synthesis of the whole group of the 4-nitro derivatives of quinoline, that this nitro radical is chemically very active and can easily undergo substitution reaction with nucleophilic substances, and we also referred to the demonstration by Okabayashi (3) of the substitution of the nitro radical with SH-group.

A series of experiments has since been carried out, and now it has become

* Preliminary announcement of this study was made before the 16th annual meeting of the Japanese Cancer Association held in Osaka, October 31, 1957 (Gann, vol. 48, 568, 1957)

evident that our early suspicion was well founded. We found that [1] NO_2 radical at the position 4 is absolutely necessary for the carcinogenicity of 4-nitroquinoline N-oxide, and the derivatives lacking this radical are non-carcinogenic, that [2] O atom attached to N also has an important rôle to play, and that [3] the derivatives of 4-nitroquinoline N-oxide with C_2H_5, CH_3 or Cl at the position 2 or 6 show the carcinogenicity of about the same order of magnitude as the original substance. The purpose of the present paper is to describe the details of the experiments which yielded these results.

Material and Methods

The following eight substances were selected for the present series of experiments from the large number of quinoline derivatives prepared by one of us (Sakai). This selection was made with view to elucidating the possible relation between the carcinogenicity and chemical structure.

1. 4-Nitroquinoline N-oxide [I], To serve as the standard for comparison of carcinogenicity.
2. 4-Nitro-2-ethylquinoline N-oxide [II].
3. 4-Nitroquinaldine N-oxide [III].
4. 6-Chloro-4-nitroquinoline N-oxide [IV].

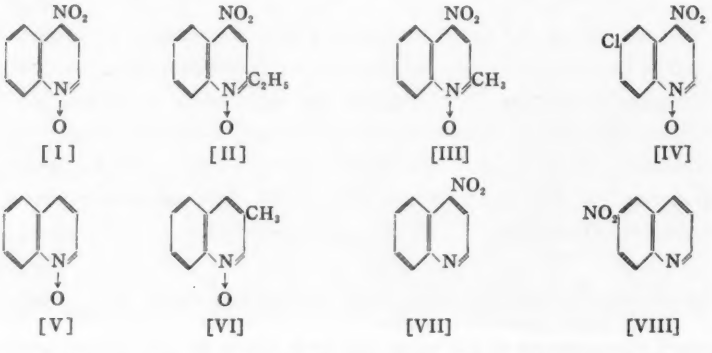
The above three to test the effect of adding CH_3, C_2H_5 or Cl radical to the molecule.

5. Quinoline N-oxide [V].
6. 3-Methylquinoline N-oxide [VI].

The above two to test the effect of the absence of the NO_2 radical.

7. 4-Nitroquinoline [VII].
8. 6-Nitroquinoline [VIII].

These last two to test the effect of the absence of the O atom.



[I] [II] [III] [IV]

[V] [VI] [VII] [VIII]

All the substances were used as 0.25 percent solution in benzene. Normal males

of a mixed strain mice, initially weighing 16 to 18 g, were used in all the experiments. Hair was pulled off over a small area of the skin at the interscapular region, and approximately 1/25~1/50 cc of the quinoline solution was applied on the depilated area by means of a small glass pipette. The treatment was repeated three times a week, discontinuing only when the tumor produced reached a sufficient size to warrant the clinical diagnosis of malignancy.

In the case of the non-carcinogenic derivatives (see the description of the results) there was a re-growing of hair in the treated area of the skin, which showed no apparent reaction. In such cases no attempt was made to pull off the re-grown hair and the treatment was continued on the hair-covered area. All the mice were maintained on the identical living conditions throughout the entire experimental period. The diet used was whole wheat grains supplemented with dried fish and fresh carrots.

A careful autopsy was performed on all the mice as they died. Some of the mice were killed when they were found in very weak physical state. In every case the macroscopical diagnosis of tumors was confirmed by histological examination. Transplantability of the tumors produced was not tested, nor was the capacity of their anaerobic glycolysis examined in this series of experiments, as there is no reason to suspect that these tumors may be different from our previous series which we adequately investigated in these respects.

Experimental Results

Early in the course of experiments it became quite evident that the substances under test sharply divided themselves into two groups according to the local reaction which they produced in the skin.

The first group consisted of 4-nitroquinoline N-oxide [I] and its 2-ethyl [II], 2-methyl [III] and 6-chloro [IV] derivatives. In each case there was a definite suppression of the re-growing of the hair in the treated skin area, which became more or less alopecic and sometimes slightly dry and scaly. In these cases papillomatous growths later began to appear in the area, and many of the growths eventually progressed to the malignant state.

Under the second group came the remaining four substances, which are without either NO_2 at the position 4 [V, VI] or N→O [VII, VIII]. The application of these substances produced no macroscopically observable reaction. Hair was soon seen to grow back, and in spite of the repeated applications for a long period of time the skin area remained normal to all appearances.

4-Nitroquinoline N-oxide (Chart 1, [I]). Six mice survived in this group to be counted as effective experimentally. The earliest papilloma was observed on the 80th day, and the first carcinoma on the 140th day after the beginning of the

treatment. At the 150 day period all the mice then surviving showed neoplastic growths, some already malignant. The tumors in Mouse 6 which died on the 187th day showed metastases to the lung.

The rate of carcinogenesis in this grop thus approximately coincided with the result of our first experiment which we reported previously.

4-Nitro-2-ethylquinoline N-oxide (Chart 1, [II]). One papilloma developed before 100 days in this group of 7 effective mice. At the 150th day period there was one mouse which did not show papilloma. This mouse was in poor physical conditions and it died before 180th day without papilloma. Papillomata which developed in 2 mice before the 150th day failed to become malignant, although they lived 190~200 days.

4-Nitroquinaldine N-oxide (Chart 1, [III]). The effective number of mice in this group was 7. The earliest papilloma was observed on the 70th day. By the 150th

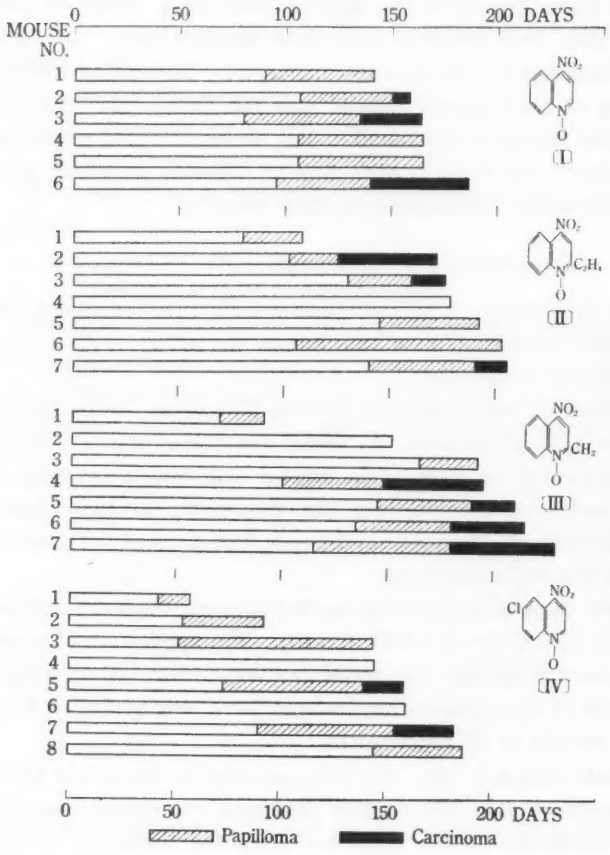


Chart 1. The rate of tumor production in 4 groups of mice treated percutaneously with 0.25 percent solution in benzene of 4-nitroquinoline N-oxide, 4-nitro-2-ethylquinoline N-oxide, 4-nitro-2-methylquinoline N-oxide, and 6-chloro 4-nitroquinoline N-oxide. The treatment was repeated 3 times a week.

day all but 2 mice showed papilloma or carcinoma, and one of these 2 mice soon died free of papilloma, the other died on 192nd day with papilloma. Altogether 4 mice produced malignant tumors in this group of 7 mice. Mouse 4 showed lung metastasis.

6-Chloro-4-nitroquinoline N-oxide (Chart 1, [IV]). Eight mice are charted in this group. There was a strong tendency in this group to show neoplastic changes earlier than in other groups, and papillomatous growths developed in as many as 3 of the mice at about 50 days after the beginning of the treatment. At the 100 day period, however, 3 mice were still without papilloma, and at the 150 day period 1 of the 4 mice then living was free from papilloma. These irregularities seem to indicate that one must be caustious in regarding the exceptionally early papilloma production in some of the mice as evidence of the specially stronger carcinogenicity of 6-chloro-4-nitroquinoline N-oxide than other derivatives. Pending further experiments with larger number of animals, it may not be warranted to state that this substance is more powerful than the other derivatives.

Quinoline N-oxide, 3-methylquinoline N-oxide, 4-nitroquinoline and 6-nitroquinoline. As already stated, mice treated with any one of these four substances showed no local reaction at the site of treatment that can be recognized in gross. In spite of repeated applications hair soon regrew in the treated area of the skin, which remained normal to all appearances for the entire experimental period. In a few cases the area became more or less alopecic after 150–200 days, but there was no sign of any local thickening or of scaliness, indicating that the continuation of the treatment for any reasonable length of time could be expected to yield a papilloma, to say nothing of malignant tumor.

Experiments were discontinued at the end of 250 days. At this period there were 2 mice surviving in quinoline N-oxide group, 3 mice in 3-methylquinoline N-oxide group, 4 mice in 4-nitroquinoline group, and 3 mice in 6-nitroquinoline group. In all these mice there was not even the faintest indication of any lesion in the treated area of the skin. The general physical conditions were excellent, and autopsy revealed no significant gross pathological change in any of the internal organs.

It must be pointed out that the possibility cannot be absolutely excluded of any of these substances eventually producing papilloma in a small percent of the mice if they are treated more vigorously (say, every day, instead of three times a week) and for far beyond 250 days. It may be recalled that Takizawa (4) obtained a few papillomas with certain quinones under just such drastic experimental conditions. We feel, however, that it is entirely justifiable to conclude that the four quinoline derivatives tested are non-carcinogenic, at least in comparison with 4-nitroquinoline N-oxide and its derivatives with which malignant tumors can be produced

with relative ease.

Pathological Notes

The course of events at the site of the application of carcinogenic quinolines was generally the same as we described in our previous paper (1). The present experiments showed, however, a few cases in which the early neoplastic processes took the form of down-growth, instead of protruding papilloma, a condition which may be styled "inverted papilloma" (Plate XIII, Figure 1). Such a growth as this does not develop the usual piling-up of hornified material on top, but tends rather to show surface erosin and ulceration. With these isolated exceptions the tumors produced were of the same general characters as those already described in the case of 4-nitroquinoline N-oxide, regardless of the difference in chemical structure of the derivatives used.

The majority of the malignant tumors consisted of squamous cell carcinoma and a small proportion belonging to the type which simulates sarcoma. These latter were referred to as sarcomas in our previous report. In view of what appears to be transitional histological picture, which can be observed in many cases (Plate XIII, Figure 2) it is probable that some of these "sarcomas" may more properly be called spindle cell carcinomas. We alluded to the difficulty in determining the cell type in many cases of our previous series of 4-nitroquinoline N-oxide tumors.

Metastases to the lung were observed in 2 cases of the malignant tumors produced in the entire series of experiments. These were a single case each from the 4-nitroquinoline N-oxide treated group (Mouse 6 which died 187 days after the beginning of the treatment) and from 4-nitroquinaldine N-oxide group (Mouse 4, which died on the 197th day). In both cases the primary tumors were squamous cell carcinoma of very large sizes, and the lung metastases corresponded to them in their histological characters. One of these lung metastases is shown in Plate XIII, Figure 3. Lymphnode metastasis was not detected in any of the present series of tumors.

It seems probable that if the mice survived longer after the development of malignant tumors many more cases of metastases would have been found.

Discussion

The results of the above described experiments are clean-cut. All the derivatives of 4-nitroquinoline N-oxide tested proved to be distinctly carcinogenic, contrasted to the lack of carcinogenicity on the part of closely related compounds which are without the NO_2 radical at the position 4 or O atom at the position 1. The absolute necessity of the presence of these two components for the carcino-

genic activity of this class of molecules may be considered as established beyond doubt. The respective role of the NO_2 radical and O atom in determining the carcinogenicity will be discussed in our next publication.

It may be of interest to note here that, according to the report by Sakai *et al.* and Fukuoka (5), the derivatives now found to be carcinogenic are more or less strongly cancericidal *in vitro*, while those that proved to be non-carcinogenic are all without such effect on cancer cells. This apparent parallelism between anti-cancer and carcinogenic actions manifested by these quinolines may be of interest in connection with the well know postulate of Haddow (6) that the substances inhibiting cancer growth may be expected to be also carcinogenic, and *vice versa*. The fact that the parallelism between these two actions is not quantitative in the vast majority of substances indicates, however, that the mechanism involved may not be the same. The discrimination between the anti-cancer and cancer producing mechanisms should form an important problem for future investigation.

In discussing the possible biochemical mode of action of 4-nitroquinoline N-oxide we previously referred to the findings of Fukuoka, Sugimura and Sakai (7) that it produces a marked inhibition of the glycolytic activity of cancer cells *in vitro*. It is of interest that the derivatives now found to be carcinogenic all show similar inhibition, while those others, proved to be non-carcinogenic, are all without this inhibiting action on glycolysis. The possibility of cells, under the stress of glycolysis-inhibiting influence, developing high glycolytic function in the manner of reactive adaptation cannot be lost sight of.

However, the ability of glycolysis inhibition cannot be generally correlated to carcinogenicity. A specially interesting case in point is that of alkylthio derivatives of 1,4-naphthoquinone, which are endowed with marked cancericidal action, paralleled with a strong inhibiting action on glycolysis of cancer cells (7). In spite of the fact that in these biological activities alkylthio-1,4-naphthoquinone does not fall behind 4-nitroquinoline N-oxide, our carcinogenesis experiments (skin painting of benzene solution on mice) with 2-methylthio-1,4-naphthoquinone demonstrated conclusively that the substance definitely belongs to non-carcinogenic category. None of the several mice that survived over 200 days produced any skin lesion suggestive of papilloma. Although the possibility is not excluded that longer and more energetic treatment may eventually yield some papillomas, as in the case of Takizawa's (4) experiments with other quinones, our results are quite sufficient to show that the high glycolysis-inhibiting activity does not in itself constitute the biochemical mechanism of carcinogenesis.

More interesting perhaps in this connection may be the observation of Okabayashi (8) who reported the occurrence of a high rate of mutation (61 out

of 231 cultures) in *Aspergillus niger* under the influence of 4-nitroquinoline N oxide in high dilution. The mutagenically effective dilution shows, according to Okabayashi, the killing rate of 94-99.9 percent, which would mean that the overwhelming majority of the spores are killed by the treatment, and the progenies obtained from the few surviving spores yield many mutant strains.

4-Nitroquinoline N-oxide produces a certain amount of local tissue necrosis when injected subcutaneously, even in concentration as low as 0.1 percent (in propylene glycol). The necrotizing effect is not so marked when applied on the skin, but we did observe obvious signs of it in isolated cases in our skin painting experiments using 0.25 percent solution in benzene. It seems possible that the cancerous transformation of cells may take place amidst the local tissue necrosis, as in the case of the mutation in *Aspergillus*. It seems not unreasonable that tissue derangement incidental to local necrosis may provide the environmental conditions favorable to the eventual production of a malignant tumor. Whether or not the primary cause of carcinogenesis is to be considered in association with the mutagenic action of 4-nitroquinoline N-oxide remains an open question. At the present time we are not willing to take a definite stand, either for or against, as to the somatic mutation theory of cancer causation.

Summary and Conclusions

In continuation of our previous experiment which demonstrated the powerful carcinogenic activity of 4-nitroquinoline N-oxide, we tested the carcinogenicity of eight closely related derivatives, based on the classical Tsutsui method. It was found that 2-ethyl, 2-methyl, and 6-chloro derivatives of 4-nitroquinoline N-oxide were about as potent as the original compound, but that quinoline N-oxide, 4-nitroquinoline and 6-nitroquinoline were non-carcinogenic.

An important conclusion was therefore arrived at that NO_2 radical at the position 4 and O atom at the position 1 are both essential to the carcionogenicity of this class of molecules.

The respective role of these two components of the molecule in determining the cacinogenicity was reserved for consideration in our future investigation. The known biochemical and biological actions of 4-nitroquinoline N-oxide were discussed in connection with the carcinogenicity of this substance.

We wish to acknowledge our indebtedness to Dr. Kunio Oota for his kindness in going over much of our pathological material.

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Explanation of Figures (Plate XIII)

Figure 1. Inverted papilloma. Mouse 3, treated with 6-chloro-4-nitroquinoline N-oxide. 144th day.

Figure 2. A part of the tumor in Mouse 7, treated with 4-nitroquinaldine N-oxide, showing what appears to be transition from squamous cell to spindle cell carcinoma. 197th day.

Figure 3. Lung metastasis of squamous cell carcinoma. Mouse 6, treated with 4-nitroquinoline N-oxide. 187th day.

要　　旨

キノリン誘導体の癌原性と化学構造との関係

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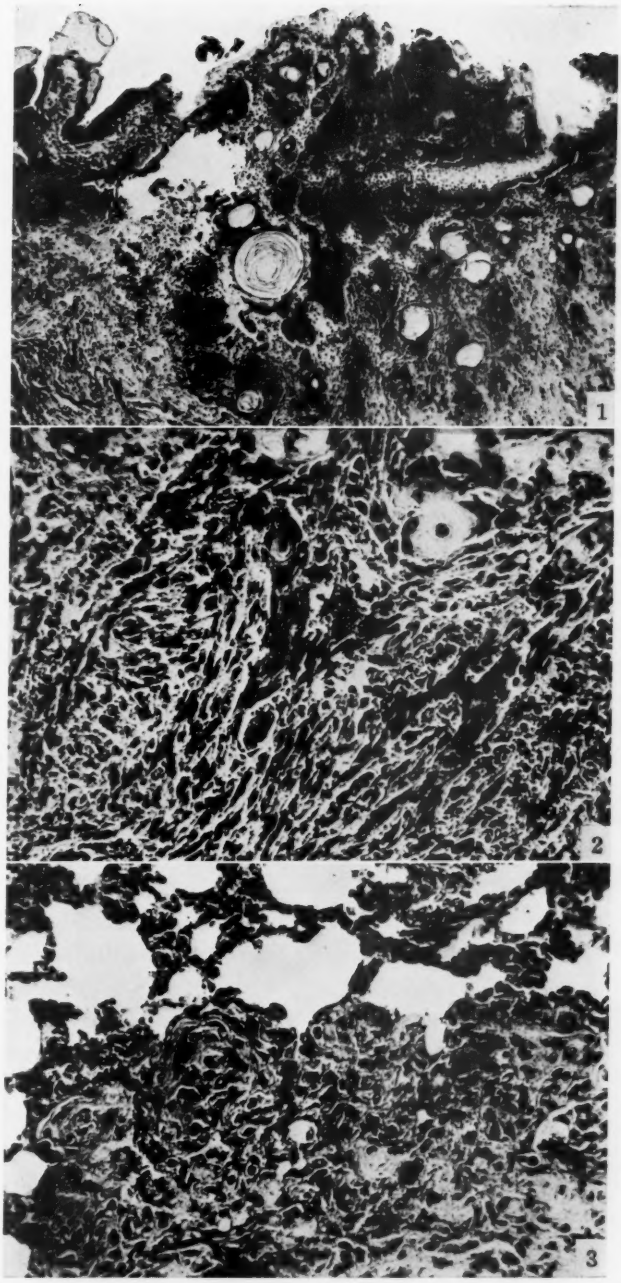
酒井純雄　(科研化学株式会社)

4-Nitroquinoline N-oxide が極めて強力な癌原性を有することは前報に発表した。今回はそれに近似の8種の誘導体について発癌実験（簡井法）を行い，化学構造と癌原性との関係を検討し (1) 4-nitroquinoline N-oxide の 2 (あるいは 6) 位に CH_3, C_2H_5 あるいは Cl 基を導入しても物質の癌原性に大なる変化は起らないが，(2) 4 位の NO_2 基と1位の O 原子とは絶対必要で，そのいずれかを欠くと癌原性が失われることを見出した。

このような簡単な二核化合物の強力な癌原性が，この NO_2 基と O 原子とによって規定されているという知見は，われわれの展開しつつある発癌機構論の具体的な出発点を成すものである。

(文部省科学研究費による)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[GANN, 49, 43~48; March, 1958]

ON THE ANALOGY BETWEEN CARCINOGENIC COMPOUNDS AND PLANT GROWTH COMPOUNDS IN THE MECHANISM OF THIER BIOLOGICAL ACTIONS

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The present authors have studied the relation between the electronic structure and the carcinogenic activity of the aromatic compounds and have found that the electronic distributions of the two regions in the molecule specified by us as the principal carcinogenophore and subsidiary carcinogenophore and the carcinogenic activity of these compounds have a distinct parallelism (1). The present authors have also calculated the electronic structure of a number of plant growth compounds, such as benzoic acid derivatives, and have found that the electronic distributions of some special positions in the molecule are intimately correlated with the plant growth activity (auxin activity) of these compounds (2).

In the present paper the electronic distribution of the plant growth compounds is tabulated and the strong resemblance between the carcinogenic compounds and the plant growth compounds with respect to the reaction center and the mode of reaction is pointed out and its meaning is discussed.

Result and Discussion

The method used in the calculation is the frontier electron theory which has been established by the present authors but in this paper we do not refer to the details of the theory (3, 4, 5, 6, 7). The theoretical indexes for discussing reactivity of a certain position (r) in the molecule, which are used in the frontier electron theory, are the frontier electron density (f_r), superdelocalizability (S_r), and the approximate superdelocalizability (S'_r). They have proved them by a comprehensive examination to be good indexes for discussing the chemical reactivity of aromatic compounds.

It should be stressed here that the S'_r is not a mere approximate to S_r, but serves as a better index than S_r in some special reactions. For example, the carcinogenic activity of polycondensed aromatic compounds has been more successfully interpreted by the value of S'_r than by S_r itself at the principal carcinogenophore and the subsidiary carcinogenophore (1). In the same way, the auxin activity of the plant growth substance shows a distinct parallelism with the values of S'_r at the ortho position in the benzene ring (2). In Table 1, the values of

Table 1. Relation Between Reactivity Indexes and Auxin Activity of Benzoic Acid Derivatives

Benzoic acid derivatives	$S'_r{}^{(N)}$	$S'_r{}^{(E)}$	$S'_r{}^{(R)}$	Net charge at the oxygen atom in carboxyl group, Q_{oxy}[b]	Auxin activity[c]
	[a]	[a]	[a]		
2, 5-Dichloro-	0.7771 (2)	0.2985 (6)	0.5239 (2)	——	++
2, 4-Dichloro-	0.7186 (2)	0.2894 (6)	0.4911 (2)	——	−
2, 3, 6-Trichloro-	0.7108 (6)	0.2753 (2)	0.4848 (6)	——	++++
2-Chloro-	0.7093 (2)	0.3043 (2)	0.5012 (2)	−0.8140	+
2, 3, 5-Trichloro-	0.7074 (2)	0.2503 (6)	0.4773 (2)	——	++++
2, 4, 6-Trichloro-	0.6918 (2, 6)	0.3137 (2, 6)	0.5028 (2, 6)	——	−
2-Bromo-	0.6825 (2)	0.3014 (6)	0.4885 (2)	−0.8150	+
2-Iodo-	0.6810 (2)	0.2942 (6)	0.4807 (2)	−0.8145	±
2-Bromo-6-Chloro-	0.6776 (6)	0.2819 (2)	0.4781 (6)	——	+
3-Methyl-	0.6705 (2)	0.4873 (2)	0.5789 (2)	−0.8157	−
2, 6-Dichloro-	0.6693 (2, 6)	0.2593 (2, 6)	0.4644 (2, 6)	——	+
2, 6-Dibromo-	0.6501 (2, 6)	0.2706 (2, 6)	0.4604 (2, 6)	——	−
2-Methyl-	0.6483 (6)	0.3384 (2)	0.4783 (6)	−0.8201	−
3-Chloro-	0.6430 (6)	0.3202 (2)	0.4691 (6)	−0.8157	−
3-Fluoro-	0.6364 (2)	0.4680 (2)	0.5522 (2)	−0.8157	−
3-Hydroxy-	0.6351 (2)	0.3188 (2)	0.4770 (2)	−0.8157	−
3-Bromo-	0.6327 (6)	0.3267 (2)	0.4662 (6)	−0.8157	−
3-Iodo-	0.6312 (6)	0.3173 (2)	0.4574 (6)	−0.8157	−
4-Chloro-	0.6199 (2, 6)	0.2982 (2, 6)	0.4590 (2, 6)	−0.8141	−
4-Iodo-	0.6160 (2, 6)	0.2874 (2, 6)	0.4517 (2, 6)	−0.8144	−
4-Bromo-	0.6142 (2, 6)	0.2888 (2, 6)	0.4515 (2, 6)	−0.8150	−
2-Hydroxy-	0.6133 (6)	0.0591 (6)	0.3362 (6)	−0.8227	−
2-Fluoro-	0.6096 (6)	0.3127 (2)	0.4526 (6)	−0.8170	−
3, 4, 5-Triiodo-	0.6079 (2, 6)	0.2487 (2, 6)	0.4283 (2, 6)	——	−
Benzoic acid	0.6020 (2, 6)	0.3289 (2, 6)	0.4655 (2, 6)	−0.8157	−
2-Amino-	0.6010 (6)	0.4555 (2)	0.4088 (6)	−0.8201	−
4-Amino-	0.5733 (2, 6)	0.4756 (2, 6)	0.5245 (2, 6)	−0.8199	−
4-Nitro-	0.5567 (2, 6)	0.3407 (2, 6)	0.4487 (2, 6)	——	−
4-Fluoro-	0.5564 (2, 6)	0.2432 (2, 6)	0.3998 (2, 6)	−0.8169	−
4-Hydroxy-	0.5538 (2, 6)	0.0825 (2, 6)	0.3181 (2, 6)	−0.8220	−
4-Methyl-	0.5427 (2, 6)	0.2488 (2, 6)	0.3958 (2, 6)	−0.8195	−
2-Nitro-	0.3764 (6)	0.3068 (6)	0.3416 (6)	——	−

a Figures in parentheses indicate the position of attack. The position of attack is assumed to be ortho with respect to the carboxyl group. If there are two non-eqivalent ortho positiions in a molecule, the one with the greater S'_r than other is chosen.

b Calculation of Q_{oxy} for di- and tri-substituted compounds as well as nitro-compaunds has not been carried out, because of it tediousness.

c The signs, + and −, indicate the degree of auxin activity. The larger the number of + sings, the greater is the activity. The sign, ±, indicates that the compounds is very feebly active. The sign, −, indicates that the compounds has been reported to be inactive.

S'_r and the auxin activity of benzoic acid derivatives are given. $S'_r{}^{(E)}$, $S'_r{}^{(R)}$ and $S'_r{}^{(N)}$ are the approximate superdelocalizabilitis for the electrophilic, radical, and nucleophilic reactions respectively. It is clearly seen that the larger value of $S'_r{}^{(N)}$ a compound has, the more active it is. On the contrary, the values of $S'_r{}^{(E)}$ and $S'_r{}^{(R)}$ have no such distinct relation with the auxin activity. Since, $S'_r{}^{(N)}$ is an index for discussing the reactivity in nucleophilic substitution, it may be said that the first step of the ***in vivo*** reaction of these compounds is the displacement of the atom or group at the ortho position by the nucleophilic group of the plant substrate. This conclusion is just the same as that of Hansch and Muir (8, 9). And the nucleophilic nature of the reaction has practically been verified by Hansch *et al.*, who measured the amount of Cl^- ion in the experiment of elongation of coleoptile section by using the 2, 6-dichloro- and 2, 4-dichloro- benzoic acids (8). They showed that the amount of Cl^- ion released is paralleled with the auxin activity of these compounds.

The discussion so far relates only to the reactivity of the ortho position in the ring, but we must pay the attention to another center, the carboxyl group. From the experiments it has been concluded that the presence of a carboxyl group in a molecule is a necessary condition to the occurrence of the auxin activity (10). In fact, any molecule with neither carboxyl group nor group convertible to carboxyl has no activity. In these circumstances many attempts have been made in order to elucidate the role of carboxyl group in the auxin action. For example, Hansch *et al.* (8, 9), from their experiments, proposed the two point attachment hypothesis (ortho position in a ring and a carboxyl group), and Wain and Schoot (11, 12) insisted that the three point attachment (ortho position in a ring, a carboxyl group and one hydrogen atom on the α-carbon atom in the side chain) will occur in the early stage of reaction. The present authors also recognize the necessity of the presence of carboxyl group, but assume that the role of the presence of carboxyl group in the auxin action is not a principal but only a subsidiary because, as is clear in Table 1, the net charge of the carboxyl oxygen atom, Q_{oxy}, has no evident correlation with the auxin activity. After Hansch and Muir, (8, 9), therefore, the present authors also suggested that the presence of the carboxyl group might be essential for the auxin activity in the sense that the function of this anionic group would be to allow the molecule approach to the plant substrate through their interaction with some cationic groups, such as amino, in the substrate, so that the ortho position of the compound might easily be subjected to a nucleophilic attack by another site of the plant substrate. In these respects, we named the ortho position and the carboxyl group as the principal auxinophore and the subsidiary auxinophore, respectively, and these correspond to the principal and subsidiary carcinogenophore in the carcinogenic

aromatic compounds respectively. As for the nucleophilic site of the plant substrate, a sulfhydryl group is most probable on account of various experimental facts (13, 14).

Such a circumstance as pointed out above bears a strong resemblance to the carcinogenic action of aromatic compounds. That is, Miller (15) and Heidelberger (16), have suggested from their experiments that the production of cancer by carcinogenic aromatic compounds might be originated from the combination of these compounds with some protein or enzyme in tissues or cells. Moreover, the site of reaction in such protein has been supposed to be the sulfhydryl group (17–21). The sulfhydryl group is considered to be easily ionized to RS^- in the animal or plant tissues. That is, the sulfhydryl group is nucleophilic under physiological conditions. Therefore, it would not be premature to assume that the carcinogenic aromatic compound combines with a nucleophilic center. This assumption is supported indirectly by the following facts: that is, Ross *et al.* (22) concluded from their extensive *in vitro* experiments that alkylating agents such as nitrogen mustard and sulphur mustard which are carcinogens as well as anticancer agents, would make a chemical combination with some nucleophilic center in the body. Furthermore, recently Nakahara *et al.* (23) discovered a remarkable fact that 4-nitroquinolin-N-oxide has a potent carcinogenic activity and this compound was found by Okabayashi (24) to react easily with nucleophilic reagent such as sulfhydryl group in a test tube.

The analogy between the plant growth compounds and carcinogenic aromatic compounds is found also with respect to their reaction centers, or the steric circumstances. As to the reaction center of the plant growth compounds, as is previously stated, two point and three point attachment hypotheses have been proposed. Similarly in regard to the reaction center of carcinogens Boyland (25) and Kooyman and Heringua (26) have proposed two region hypothesis. The present authors (1) have previously supposed that the carcinogenic hydrocarbons would make a chemical combination at the principal carcinogenophore with some group of protein or enzyme, and at the same time, a subsidiary action for this interaction would be performed by the subsidiary carcinogenophore. This is strongly similar to the mechanism of hormonal action of the plant growth compounds suggested by Hansch and Muir (8, 9) and by the present authors. The resemblance in the reaction center is clearly seen in Fig 1. So far as these analogies exist, the investigation of carcinogenesis and that of the plant growth action caused by chemical substances would provide each other some effective hints or suggestions. In fact, Nickell (27) has recently considerd the cancer metabolism in connection with the auxin action of the plant growth compounds.

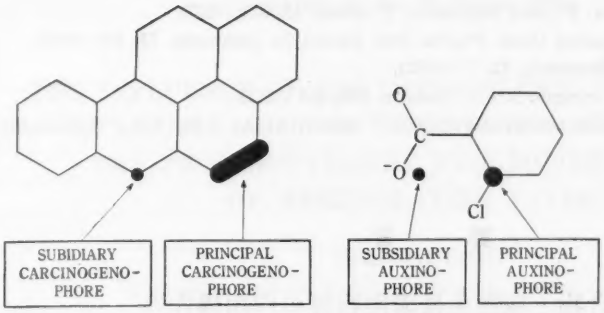

Fig. 1. Analogy of reaction centers in carcinogenic and auxin active substances.

Summary

The correlation between the electronic structure and the auxin activity of the plant growth compound is discussed. The strong resemblance between the carcinogenic compounds such as polycondensed aromatic hydrocarbons and the plant growth compounds such benzoic acid derivatives is pointed out and its meaning is discussed.

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要　旨

発癌性化合物と植物生長素性物質の作用機作の類似性について

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著者らは，さきに π- 電子をもつ発癌性物質について，著者らの提出したフロンティアー電子理論を用いて，電子状態と発癌性との関連をしらべ，その間に顕著な並行関係の存在することを見いだしたが，同じように，安息香酸誘導体のような植物生長素性物質の電子状態とホルモン活性との間にも密接な関係があることを知った。

本論文では，これらの物質の電子状態とホルモン活性との関係を簡単に述べ，これが，発癌性物質と，その作用機作および活性中心に関して極めて強い類似性をもっていることを指摘し，これらの関連を論じた。すなわち，反応様式は，生体内の求核的反応基 (ここでは SH 基) との反応であると考えられ，また反応中心としては，発癌性物質の主発癌団，副発癌団に対応して植物成長素性物質にも二つの活性団 (これを著者らは **Principal auxinophore**, および **Subsidiary auxinophore** とよんだ) が考えられたが，これらの作用機作は極めてよく類似している。

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THE INFLUENCE OF THE INTRASPLENIC IMPLANTATION OF NICOTINAMIDE ON THE MITOTIC ACTIVITY AND THE PHOSPHOPYRIDINE NUCLEOTIDE CONTENT OF REGENERATING LIVER*

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It has repeatedly been reported (1-7) that the level of total pyridine nucleotide (PN) is generally lower in various neoplastic tissues than in normal mouse or rat tissues. This decrease of PN level may be caused by either an increase in the rate of its destruction or a decrease in the rate of its synthesis. The destruction of diphosphopyridine nucleotide (DPN) may be caused by the action of several enzymes (8), but the inhibition of it by nicotinamide suggests that its breakdown is largely due to the action of DPN nucleosidase (9). However, Quastel and Zatman (10) observed that the DPN nucleosidase activity of a variety of tumours differs little from that of normal tissues. Hence it seems probable that the lowered DPN level in the neoplastic tissues is not due to the increased activity of DPN nucleosidase but to a decreased activity of DPN pyrophosphorylase, which is localized in the cell nucleus (11) and catalyzes the synthesis of DPN from nicotinamide mononucleotide and adenosine triphosphate (12). Branster and Morton (13) reported that the rate of DPN synthesis by isolated nuclei was markedly decreased in mammary carcinoma and growing foetal liver as compared with that in normal mammary gland and liver of adult mouse, respectively.

These observations suggest that the lowered PN level in such growing tissues as tumours may have some correlation with the rate of cell division which is generally very high in these tissues. On the other hand, it was reported (14, 15) that a marked increase of DPN content occurred in the liver and transplanted tumours following the injection of nicotinamide and other related substances into mice, reaching a maximum from 8 to 12 hours after the injection.

Therefore, it seems of interest to study the influence of such increase of DPN content on the mitotic activity of the growing tissue. In the present study, the effects of nicotinamide on the DPN content and the mitotic activity of regenerating rat liver were examined, nicotinamide being implanted into the spleen in the

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form of a pellet with or without polyethylene glycol (Carbowax).

Materials and Methods

Male Wistar strain rats weighing between 130 and 160 g were divided into 3 groups. In the first group, the rats received median laporatomy and a pellet of 10 mg of nicotinamide was implanted into the spleen of each individual. The animals which were traumatized on the spleen instead of implanting the pellet served as control. In the second group, the left and median lobes of liver were removed and a pellet of 10 mg of nicotinamide was implanted into the spleen. The animals which were partially hepatectomized and traumatized on the spleen without receiving the pellet served as control. In the last group, the rats were partially hepatectomized as in the second group and received a pellet containing 10 mg of nicotinamide and 10 mg of polyethylene glycol (Carbowax 6000: Carbide & Carbon Chemicals Co.,) into the spleen. The pellet was made as follows: 100 mg of polyethylene glycol was gently warmed to a melting state and mixed with 100 mg of nicotinamide. The mixture was cooled and a proper portion of it was made to a pellet weighing 20 mg. The animals which were partially hepatectomized and received 10 mg of polyethylene glycol into the spleen served as control. All the animals were starved for 24 hours after the operations but supplied with water *ad libitum* and sacrificed at the end of this period.

The PN content was determined by the method of Feigelson, Williams and Elvehjem slightly modified (16). The animals were decapitated and livers were rapidly perfused with 2 ml of ice-cold isotonic NaCl solution by injecting through the portal vein to remove the bulk of the blood. This procedure was performed within a few minutes. The liver was then removed quickly and about 1 g of the tissue was weighed on a torsion balance and dropped into a glass homogenizer containing 2 ml of ice-cold 10 per cent trichloroacetic acid and 0.5 ml of 30 per cent hydrogen peroxide. The tissue was then homogenized at 0°C for a minute, and after the addition of 8 ml of deionized water and 1 ml of 2 per cent trichloroacetic acid, homogenized again for a minute. The homogenate was allowed to stand for 10 minutes at room temperature and centrifuged at 3500 r. p. m. for 5 minutes. 10 ml of the supernatant was poured into a charcoal column which had been prepared as follows. 150 mg of acid-washed and dried charcoal (Merck) was put into a burette, 0.8 cm in diameter and 10 cm in length, with a reservoir on the top, over a tightly packed glass wool plug and covered by another thin glass wool plug. Air pressure was usally applied to the column to accelerate the rate of flow. Immediately before use, approximately 10 ml of 2 per cent trichloroacetic acid was rapidly drawn through the column under pressure. When the level of trichloroacetic acid approached the top of the charcoal, the pressure

was removed and the solution to be adsorbed was poured into the column. The supernatant was allowed to flow through the column at the rate of about 15 drops per minute under an adequate pressure. Following complete passage of the supernatant through the column, 10 ml of 2 per cent trichloroacetic acid was added to remove any non-adsorbed impurities and drawn rapidly. Then 11 ml of 10 per cent pyridine was added and allowed to flow at the same rate as in the adsorption. The eluate was collected in a graduated cylinder. To 5 ml of the eluate in a test-tube was added 1 ml of 0.5 m $NaHCO_3$. To another test-tube containing 5 ml of the eluate were added 0.4 ml of 0.5 M $NaHCO_3$ and 0.6 ml of freshly prepared $Na_2S_2O_4$ solution (100 mg of $Na_2S_2O_4$ in 5 ml of 0.5 M $NaHCO_3$) as reducing agent. 5 ml of 10 per cent pyridine in each of two test-tubes was treated as above. All the tubes were placed in a boiling water bath for 90 seconds, and then cooled in an ice bath and kept there through the following procedure. 1 ml of 0.5 M Na_2CO_3 was added to all the tubes and oxygen was bubbled through for 2.5 minutes. The solutions were read in a Shimazu spectrophotometer at 340 mμ in a 1 cm quartz cuvette against deionized water. The difference in optical density between the oxidized and reduced samples was regarded as due to the reduced PN, correction being made for the blank value in pyridine solution. A value of 6.22×10^3 at 340 mμ was used for the molar extinction coefficient of reduced PN (17).

Another piece of tissue weighing about 150mg was weighed and homogenized in 3ml of 10 per cent trichloroacetic acid for 2 minutes and centrifuged at 3,500 r.p.m. for 5 minutes. The residue was extracted again as above and the residue was suspended in deionized water and its volume was adjusted to 10 ml. 0.5 ml of the suspension was used for the determination of nitrogen by micro-Kjeldahl method. The obtained value of protein N was used as the standard for calculation.

For the estimation of mitotic activity, small and thin pieces of tissue were cut from the middle part of right lobe of liver and fixed in alcoholic Bouin's solution over 5 hours, embedded in paraffin and sectioned at 5 μ. The sections were stained by Meyer's acid haematoxylin and eosin. First, the average numbers of total nuclei in one field of microscope were determined, and the count of the mitoses was taken using 100 fields. Then the values were expressed as numbers of mitoses per 10^5 nuclei.

Results

Attempt was first made to examine the effects of intrasplenic implantation of 10 mg of nicotinamide on the mitotic activity and the level of PN content of the intact liver, using rats which had received median laporatomy only. As shown

Table 1. The effects of implanted nicotinamide on the PN content and the mitotic activity of intact rat liver.

Treatment	PN content mg/100mg of protein N	Mitosis/10^5 nuclei
Median Laporatomy and Implantation of 10 mg of Nicotinamide	1.87	0
	2.16	0
	2.63	0
	2.56	0
	2.11	9
(average)	2.27	2
Median Laporatomy and Traumatization	1.97	7
	2.29	15
	2.71	3
	1.90	3
	2.65	0
(average)	2.30	6

in Table 1, mitotic activity was generally very low in intact livers whether nicotinamide was implanted or not. However, it is to be noted that in rats receiving the nicotinamide practically no mitoses were found at all, whereas in control rats the average number of mitoses per 10^5 nuclei was about 6. This result obtained from the control untreated rats is in good agreement with that found in the liver of normal rats by Friedlich-Freksa and Zaki (18). It seems that the mitotic activity of the liver was not affected by such operations as median laporatomy or traumatization on the spleen alone. With respect to the PN content of the intact liver, no appreciable difference was found between nicotinamide-implanted and control rats. The PN content was 2.27 mg per 100 mg of protein N in the former and 2.30 mg in the latter on an average (Table 1).

Experiments were then performed using regenerating rat liver. When a pellet of 10 mg of nicotinamide was intrasplenically implanted in rats after partial removal of the liver, the PN content of the regenerating liver was markedly increased after 24 hours, the average being 4.18 mg of PN per 100 mg of protein N. The PN content of the control liver in regeneration which had received no nicotinamide was about 2.24 mg on an average. Morever it was extremely interesting to observe that on implantation of nicotinamide the numbers of mitoses were greatly reduced. Whereas the control liver in regeneration contained about 1370 mitotic figures per 10^5 nuclei, the regenerating liver of the nicotinamide-treated animals showed only 234 mitoses on an average. These results are summarized in Table 2.

The results of the experiments in which pellets containing nicotinamide plus Carbowax were implanted into the spleen after partial hepatectomy are given in Table 3. It is seen that in control rats receiving Carbowax alone mitotic activity of the liver is very high, the average number of mitoses being 1580. However,

Table 2. The effects of implanted nicotinamide on the PN content and the mitotic activity of regenerating liver.

Treatment	PN content mg/100mg of protein N	Mitosis/10^5 nuclei
Partial Hepatectomy and	7.55	6
Implantation of 10 mg	8.69	9
of Nicotinamide	2.08	917
	2.35	0
	2.38	135
	2.22	569
	4.00	0
(average)	4.18	234
Partial Hepatectomy and	1.92	4730
Traumatization	2.18	175
	2.27	0
	2.11	1180
	2.46	1290
	2.35	1820
	2.40	408
(average)	2.24	1370

Table 3. The effects of implanted pellet containing both nicotinamide and polyethylene glycol on the PN content and the mitotic activity of regenerating liver.

Treatment	PN content mg/100mg of protein N	Mitosis/10^5 nuclei
Partial Hepatectomy and	3.31	0
Implantation of 10 mg.	3.23	0
of Nicotinamide with 10mg.	3.57	0
of Polyethylene glycol	2.53	228
	3.34	16
	2.24	0
(average)	3.04	41
Partial Hepatectomy and	2.30	533
Implantation of 10mg	1.88	3140
of Polyethylene glycol	2.07	884
	1.85	284
	1.81	2470
	1.79	2190
(average)	1.95	1580

in the rats receiving nicotinamide and Carbowax together the mitotic activity is greatly reduced (41 per 10^5 nuclei). It is also striking to observe that the level of PN content is invariably increased from the control value of 1.95 mg per 100 mg of protein N to 3.04 mg on implantation of nicotinamide plus Carbowax.

Discussion

In the present study, intrasplenic implantation of a pellet was employed, so as to enable nicotinamide to exert its effects continuously and directly on the liver, and, indeed in the case of implantation of Carbowax mixture, a very clear-cut

result was obtained both as regards the PN content and mitosis. Since, however, the nicotinamide is very soluble, the observed increase in the PN content of the liver may have been only temporary. That the increased PN content following the injection of nicotinamide returns to the normal value 24 hours after the treatment has been reported (15). At any rate, the results obtained in the present study have clearly shown that PN content of the regenerating rat liver is greatly increased following the implantation of nicotinamide into the spleen, and that at the same time the mitotic activity is much depressed.

The question may be raised as to whether the inhibitory effect of nicotinamide on mitosis is due to the fact that it is generally toxic to the tissues, since it has been reported (15) that the high dose of this substance, over 2,000 mg per Kg of body weight, is very toxic to the mouse. However, the dose used in the present study was very low (approximately 70 mg per Kg of body weight) and there was no sign of degenerative changes in the liver, although doses over 70 mg were found to be slightly toxic, especially in case of partially hepatectomized animals.

On the other hand, Kaplan *et al* (15) also reported that no increase of free nicotinamide could be detected in the liver of mice after the injection of this substance and that most of the increased PN consisted of DPN.

Branster and Morton (13) indicated that the activity of DPN nucleosidase remained unchanged in the tumour and growing foetal tissues, whereas the rate of DPN synthesis was reduced. In the present study also the DPN content of the regenerating liver has been shown to be slightly lower than that of normal liver. Therefore it is possible that DPN synthetic activity is stimulated by nicotinamide implantation and that the high level of DPN is by itself responsible for the suppression of mitosis in the liver, but there is no direct evidence as to how this suppression is brought about.

In this connection the work of Waravdekar (19) is very interesting. He showed that the livers of mice bearing various forms of tumour had a reduced DPN synthetic activity as compared with livers from normal mice, and that following the surgical removal of tumour the DPN synthetic capacity restored to normal. The depression of DPN synthesis in tumour tissues may probably be due to the deficiency of some precursor substances rather than to the lack of DPN synthesizing enzymes, for it has been shown that even the tumour tissues can maintain high level of DPN, when nicotinamide is injected. There is also evidence that nicotinic acid content is generally low in the tumour than in normal tissues (20). In view of the results of the present study, it seems necessary to investigate whether mitosis in tumour tissues is also retarded in the same way by the implantation of such a precursor substance of DPN as nicotinamide.

Summary

1. When a pellet of nicotinamide (10 mg) with or without Carbowax is intransplenically implanted in rats after partial removal of the liver, PN (or presumably DPN) content of the regenerating liver is invariably increased after 24 hours, and at the same time the numbers of mitoses are greatly reduced.

2. In rats receiving a pellet of 10 mg of nicotinamide without hepatectomy, the PN content of the intact liver differs little from that of the control animals, although the mitotic activity is much reduced.

3. It was suggested that there is a correlation between the PN content of a given tissue and the rate of mitosis.

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要　旨

再生肝の Pyridine Nucleotide 及び Mitosis に及ぼす Nicotinamide の影響

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1. 肝臓の一部を切除したラッテの脾臓内に，10 mg のニコチン酸アミドを，単独で，またはカーボワックスとともにペレットにして埋植した。処理後，24時間を経ても，再生肝のピリジンヌクレオチドの含量は増加しており，同時に核分裂像が著しく減少しているのが観察された。

2. 肝臓の切除を受けぬラッテを，10mg のニコチン酸アミドで処理した場合には，処理を受けぬ対照群に比して，その肝臓のピリジンヌクレオチドの含量はほとんど変化が認められなかったが，核分裂像は非常に減少しているのが観察された。

3. 組織中のピリジンヌクレオチドの含量と，その組織の核分裂能との間の関連性について論議された。

(文部省科学研究費による)

[GANN, 49, 57~64; March, 1958]

A NEW TYPE OF VIRUS FOUND IN A SPONTANEOUS MAMMARY TUMOR OF SL MICE AND ITS PROLIFERATING MODUS OBSERVED IN ULTRA-THIN SECTIONS UNDER THE ELECTRON MICROSCOPE

(Plates XIV–XVII)

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Preface

Among the SL mice, known as a leukemia inducing strain now under our observation, a rare case of spontaneous mammary tumor was found. Like other leukemic cases, the mouse was investigated cytologically referring to the neoplastic tissues, especially utilizing ultra-thin sections under the electron microscope. Neither virus matrix nor inclusion body was found inside of these cancer cells. However, in the lumen of the duct of the cancer abundant viruses in uniform size and form with nucleoid structure were remarked. The virus proliferation took place mostly just under the cell surface, just in a mode like that which was described by Morgan *et al.* (1956) recently referring to influenza virus in chicken embryos.

The findings suggest that (a) the existence of viruses common to the so-called mammary cancer strains such as C_3H, DBA, RIII may be extended to the mammary cancers arising spontaneously in mice other than the mammary cancer strain, and furthermore, (b) an influenza type of virus proliferation should be considered hereafter to understand the distribution of the viruses in tumor tissues. These two points, we think, will give new cautions for the further research in this field.

Materials and Methods

The inbred SL mice were cultivated in the National Institute of Genetics at Mishima and transported to the laboratory of Prof. Hashimoto, which were again distributed to our laboratory.

The mouse which gave rise to a spontaneous mammary cancer is a female aged 1 year and 6 months, and is the only one cancer case in our SL mice under observation. The others belong to lymphatic leukemia or leukosarcomatosis. According to the observation of Hashimoto and Baba (1956) concerning 105 mice of SL strain,

35 cases of lymphatic leukemia or sarcoma were produced spontaneously, but no mammary cancer was observable.

Electron microscopy: Small pieces of cancer tissue were fixed in 1% osmic acid according to Palade's method and inbedded in methacrylate. Ultra-thin sectioning was carried out by Shimadsu's KS type of ultra-microtome. The electron microscope used was Hitachi's HU-10 type electron microscope.

Macroscopic and Microscopic Findings

1) **Macroscopic findings:** The tumor of a size of a bean located in the left axial region with a small ulceration on top. The tumor tissue was grayish white, hard, and was difficult to separate from the skin, though the adhesion to other sides was slight. (Photo 1) The spleen was enlarged, but the lymph nodes were not so enlarged except in the vicinity of the tumor. No enlargement of the liver.

2) **Microscopic findings:** The tumor tissue was typically adenocarcinomatous (Photo 2). Considering from the localisation, the left second mammary gland was supposed to be the site of origin. Supravital phase contrast observation as well as staining of tissue stumps and sections revealed no inclusion body in the cytoplasm of the tumor cells. Except the regional lymph node which had metastasis, no other metastasis was found. The spleen showed hyperplastic follicles and engorgement of the sinusoid with concomitant leukocyte infiltration. The local lymph nodes showed also leucocyte infiltration beside tumor metastasis. The femoral bone marrow contained no tumor metastasis.

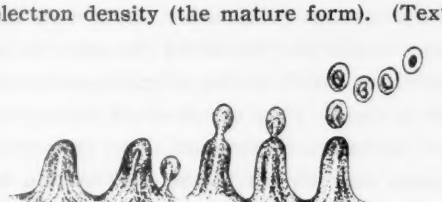
3) **Electron microscopic findings.**

Tumor cells: Nuclei are oval, the nuclear membranes, though there are small uneveness occasionally, are mostly smooth and clearly double membranous. Nucleoli: one or two in number with strong electron density. They are relatively small and regularly oval just to accord to the mode of epithelial cells, as compared with the lympholeucosarcoma cells which were produced in the same SL strain mice. Mitochondria are apt to be in swelling. Golgi canaliculi are scanty in development. Golgi granules in size of 30–50 mμ should be carefully differentiated from the viral particles, in discussing the distribution of the latters (Photo 3, 4, 10).

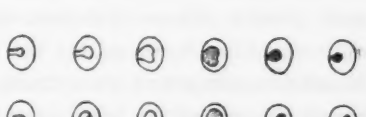
Endoplasmic reticulum is more or less scanty in most cells, but in some cells exceptionally remarkable in size and number (Photo 3). These latter are always cystically round and not lamellar, differning from the type of secretory cells, which makes us suspect the influence of the viral existence. Occasionally, we see around these distended endoplasmic reticulums a distribution of small particles in uniform size (diametrical size 35–50 mμ) in place of RNA granules (Photo 10). These small particles, in regard to their internal structure, are weak in electron

density and somewhat vacant, and therefore are suspected as immature viruses. This is the only mode of the viral distribution in the cytoplasm. However, we could not ascertain the process of the removing of these viral particles to the duct lumen. No other forms corresponding to virus matrix or inclusion body were found despite our energitic search in the ultra-thin sections.

The viral particles we encountered most often in the duct lumen are slightly larger (50–70 mμ×75–100 mμ) than those found intracytoplasmically and are oval. (Photo 5, 6, 7, 8, 9). They are classified in two types, namely (a) single membranous but with internal structure of weak electron density (the immature form), and (b) single membranous but with internal structure (nucleoid) of more or less strong electron density (the mature form). (Text-figs. 1 & 2)



Text-figure. 1. Half schematic representation of the developmental process of viral particles from the top of the microvilli of cancer cells facing to the duct lumen. 1, 2: preceding stage, 3, 4, 5: separation, 6: maturation of viral particles.



Text-figure. 2. Schematic representation of the maturation process of viral particles in the duct lumen. 1, 2, 3, 4: immature forms, 5, 6: mature forms.

The most interesting findings were the interrelationship between the microvilli of the cellular surface faced to the duct lumen and viral particles there. Inside of the microvilli we see often 2 fine filaments with moderate electron density or longitudinal striations (diametrical size ca. 25 mμ) (Photo 7, 8). In most cases the top of microvilla becomes electron dense and contains a faint corpuscle corresponding to the size of viral particle to whose center the end of the beforementioned filament arrives. This central filament may be followed to the basal part of the microvilla, where it arises from a net structure distributed to the cellular surface (Text-fig. 1). Now the top of the microvilla is constricted to a size of the viral particle, and the narrowing of the microvilla takes place at the proximal side. Thus the viral particle is separated to be free in the duct lumen with a minimal covering of cytoplasm containing a small piece of the central filament in its center as a nucleoid (Text-fig. 1.). And, according to the viral maturation, the size of this nucleoid is altered (diametrical size ca. 25 mμ → ca. 40 mμ → ca. 25 mμ) (Text-fig. 2.), while its electron density increases gradually. Occasionally, such a viral production may be found at the basal part of the

microvilla, but it generally confined to the cellular surface.

As to the epithelium of this cancer tissue, in which viral proliferation is unobservable, the microvilli are not so electron dense and are deprived of the central filaments.

Discussion

That the lactating mammary glands and mammary tumors of the so-called mammary cancer strain of mice contain milk agent viruses is verified by way of electron microscopy utilizing ultra-thin sections by Dmochowski *et al.* (1953, 1955), Bernhard (1955), Suzuki (1957). According to the report made by Dmochowski *et al.* the virus particles have the size of 50–145 mμ in diameter, each with the nucleoid structure (15–35 mμ in diameter). Suzuki also measured the size to be nearly 100 mμ. These viral particles distribute mostly in the ectoplasm and form proliferating foci in groups, but not in the nucleus. They are removed just under the cell membrane and are extruded into the lumen of the duct. The intracytoplasmic clusters of the proliferating viruses may accord to the proliferation in the matrix region which was described precisely by our collaborator (Matsumoto, 1956). The viruses extruded into the duct lumen bear the nucleoid structure. Besides, Suzuki described cytochemically an inclusion body without viral particles in the same tumor cells.

Now the SL strain mice, on the contrary, produce leukemia in ca. 30%. According to the observation of Hashimoto and Baba (1956) no mammary cancer was detected among nearly 40 cases of lymphatic proliferation (leukemia and sarcoma). We have also observed such a lymphatic proliferation as leukemia and lymphosarcoma, but the present mammary tumor belongs to an exception.

Therefore, the mammary tumor found in SL strain should be evaluated as different from those which were reported by Dmochowski, Bernhard and Suzuki.

It is the matter of common sense that the mammary cancer appearing exceptionally among the leukemic strain mice should be dealt with differently from that of the low-frequency mammary cancer strain (by which it means that the tumor other than the mammary cancer does not appear). Naturally, the former is also evaluated as different from that of the high-frequency mammary cancer strains. The analogy may be sought to the relationship between the mammary cancers of human and high-frequency mice strain. This leads one to suspect, furthermore, the possibility of finding virus particles in the human mammary cancers.

In the cancer cells we obtained in the SL mouse, neither inclusion body nor matrix for viral proliferation were detectable, though the viral excretion into the duct lumen is so abundant. However, the cytoplasmic surface facing the duct

lumen and their microvilli (and the smooth-surfaced endoplasmic reticulum communicating to the lumen occasinally) are the place for the proliferation of the viruses.

This mode of virus proliferation is analogous with that of influenza virus which was detected by Morgan *et al.* (1956), utilizing chorio-allantoic membrane of the chicken embryo. However, something is different in the detail concerning the viral composition and distribution in the cytoplasm of cellular surface.

The predominant site of the viral development is the top of the microvilli of the cancer cells facing the duct lumen. These microvilli producing viruses have two fine filaments running axially in their center, which arise from the net structure of the basal part of the microvilli. When the viral particles are produced at the top of the microvilli and separated from there a small piece of the central filaments is also contained in that viral particles as a central body or a nucleoid. This is the characteristic attitude of the viral generation in our case. Matsumoto (1957) in our laboratory observed recently in the case of the proliferation of ectromelia viruses in the azurophilic inclusion body of the Ehrlich ascites tumor cells that "subchromonema-like filamentous substance" was taken into the viral membrane to be an essential part of the internal structure of the virus. Something is common to the understanding in the formation of viral particles in the present case that the viral membrane and internal structure are constituted from the different elementary parts. Our central filament taken into the viral particle at the top of the microvilli changes afterwards into a central body or nucleoid acquiring strong electron density gradually.

This process is quite different from the simple extrusion of immature viruses from the cellular surface in the case of milk agent virus, that is described schematically by Bernhard (1957) recently.

The interrelationship between the host cell and viruses is understood generally just in a way like that between the coli bacilli and bacteriophage, in which the host enzyme system is utilized toward the phage proliferation. This is also true for the case of ectromelia virus proliferation in the Ehrlich ascites tumor cells, as we described it electron microscopically for these years (Matsumoto, 1954, 55, 56).

So far as the relationship between the tumor cells and cancer viruses is concerned, however, they should co-exist in mutual benevolence. From this viewpoint, intranuclear proliferation of the virus which disturbs the mitosis of the host cell is not possible in this case (Amano, 1957). Even the intracytoplasmic proliferation of the virus which occupys the most part of the cellular metabolism is also improbable in this case. Therefore, the third position of the viral proliferation which is realized in our present case in a way like that of influenza

virus in chorio-allantoic epithels presents a most probable mode fit for the cancer producing virus. So far as influenza virus in host cells is concerned it produces some toxic substance to disturb host cells, rather than a mere metabolic consumption. And this will not be the case with our tumor virus. On the contrary, our tumor virus will give a stimulation which is potent for the RNA formation for both host and parasite, which is presumable from the electron microscopic findings demonstrating relatively strong electron density of the cellular margine; a conjugative formation of virus and host RNA.

The size of our cancer viruses (50–70 mμ×75–100 mμ, namely ca. 60 mμ×87 mμ) is somewhat smaller than that are found in such mammary cancer strain as C_3H, DBA, RIII. However, there are some similarity in the form and internal structure of both sorts of viruses. It may also be assumed that the viral particles found in the Rous sarcoma cells (Gaylord, 1955)—in two of the three tumors tested —possiblly follow the proliferation mode of our cancer viruses.

Therefore, the present findings of the viral proliferation of our cancer cells will give a valuable suggestion to the future study in looking for the viral agent in the other tumor cells.

We have now obtained another finding concerning the leukemic and sarcoma cells of SL strain mice, which will be reported later as compared with that of the leukemic cells from the Ak mice.

Summary

1) Using a spontaneous mammary cancer arisen exceptionally rarely among the leukemic strain of SL mice, the electron microscopic observation of ultra-thin sections of tumor tissue was made.

2) The cancer tissues contain abundant viral particles (50–70 mμ×75–100 mμ) in their lumen of the duct which are similar to the viruses described in the tissues of mammary cancer strains C_3H, DBA and RIII by many authors. However, in the present case, neither viral matrix nor inclusion body in cytoplasm were found.

3) Predominant foci of the virus proliferation are the ectoplasm just under the cellular membrane facing the duct including epithelial microvilli. Therefore, the type of proliferation is almost similar to that of the influenza virus described recently by Morgan *et al.*

4) However, in our case, the virus producing microvilli have central filaments in the axial region. After the production of the viral particle at the top of the microvilli a small piece of the central filament is involved there and the separation of the virus takes place. The finding suggests the dual origin of external and internal structures of the virus.

5) The fact that the mammary cancer tissue of non-mammary cancer strain

mice such as SL mice contains viral particles allows us to further expect that the other sporadic mammary tumors may also contain such viral particles.

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Explanation of the Photo-Plates (**Plates XIV–XVII**)

(Abbreviations; N: Nucleus, E: Epithelium, Ec: Epithelial cytoplasm, Mv: Microvilli, F: Central filament, D: Duct lumen, Arrows: Viral particles.)

Photo. 1. SL strain mice No. 70, which produced the mammary cancer.

Photo. 2. Histological finding of the mammary tissue corresponding to adenocarcinoma.

Photos. 3 and 4. Ultra-thin section of the tumor tissue. Neither inclusion body nor viral matrix are observable in the cytoplasm. In Photo. 3 distended endoplasmic reticulums (s. later) are seen in groupings. In Photo. 4 a duct lumen is shown. Microvilli of the epitheliums facing the duct are seen in various densities.

Photos. 5 and 6. Ultra-thin section of the tumor duct where viral particles are abundant. Photo. 6 is an enlargement of the Photo. 5. Though the mode of viral production is not clear in this photo, the structure of viral particles having nucleoid and in uniform size is presented.

Photos. 7 and 8. Ultra-thin section of the tumor cells facing the duct lumen. Microvilli have central filaments in the axial region. When the viral particles in the top of the micro-

villi are produced, the diametrical size of this part is diminished to the size of the viral particles and proximal constriction is taken place. Thus a piece of the central filaments is contained in the viral particles separate in the duct lumen. The forms of these nucleoids of filamentous origin are varied, but they may be understood as variants of crumpled forms (see Text-figs. 1 and 2).

Photo. 9. Tangential section of the cellular surface with abundant virus proliferation. In this photo, microvilli with some larger diametrical size (with central filaments) and viral particles with young nucleoids should be differentiated.

Photo. 10. Enlargement of distended endoplasmic reticulum of Photo. 3. In place of RNA granule at the outer surface of the endoplasmic reticulum ca. 35-50 mμ particles suggesting viral generation are arranged. The finding reminds one the mode of the proliferation of milk agent virus. However, this mode is seen only occasionally in our case.

要　　旨

SL 純系マウスの特発性乳腺癌中に発見された一新ウィルス種及びその増殖態度について（超薄切片の電顕的研究）

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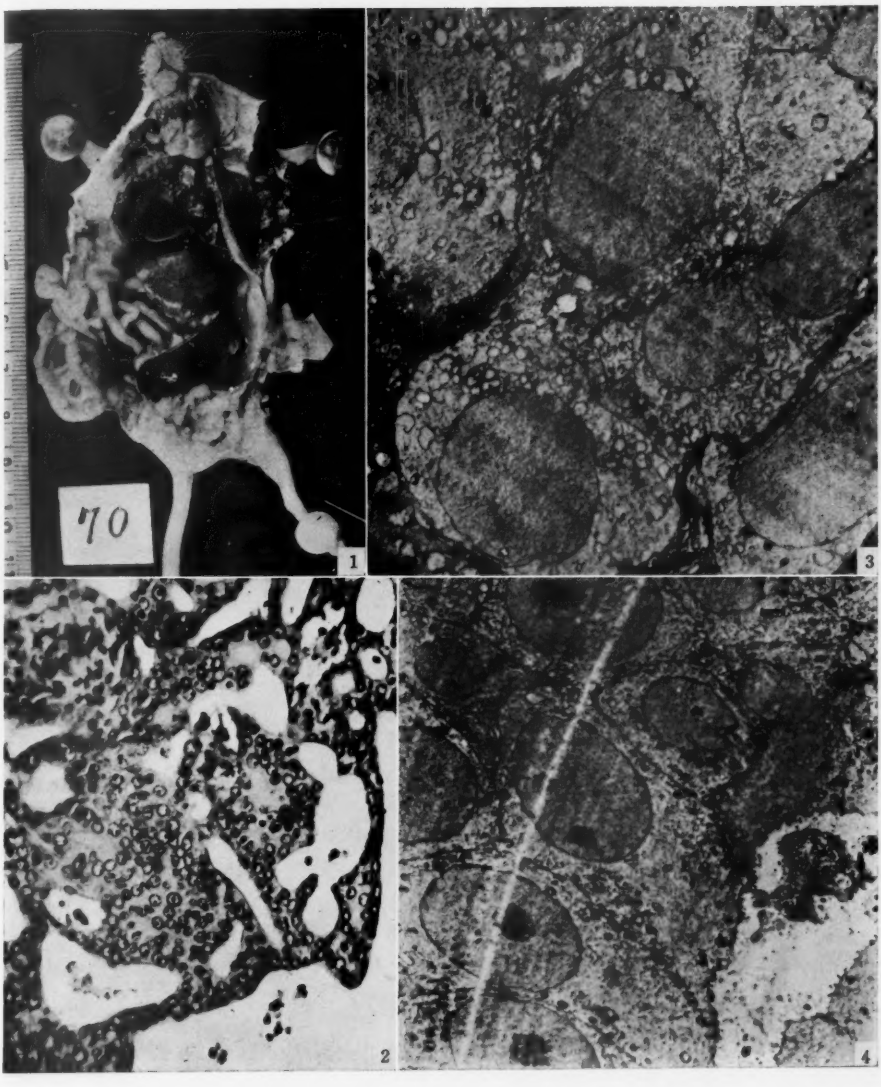
C_3H の乳癌細胞中に特有の封入体と増殖様式とを示すウィルスの発現することは，もはや，確認されているが，このような乳癌発生系に属しない系統のマウスに認められる特発性のマウス乳癌においても，同様に，ウィルスが存在するかは別の重要課題である。白血病頻発マウス SL 系の飼養観察中に，極めて稀であるが，乳癌の発生を認めて，これを電顕的に超薄切片によって観察中，形態・大さにおいて全く Milk factor 類似のウィルス(50～70mμ×75～100mμ 大にて核様物を備える）を確認した。このウィルスは癌細胞腺腔面の細胞膜直下で生じ，直ちに腔内に排出されるが，鈴木氏が C_3H, DBA 系で示した如き細胞内封入体や，細胞内増殖集団は作らない。従ってその増殖様式は Morgan 等のインフルエンザウィルス増殖の形式に従うものといえよう。しかしながらわれわれの場合，ウィルスを作る細胞小突起には軸の位置に中心繊状物があり，この中心繊状物の一端はウィルスが完成して細胞小突起の尖端から離断する際にその中に含まれ核様体となる。これは該ウィルスの外内構造の二元性を物語るものである。ウィルスが癌性増殖に関与するためには，核内増殖では不適当であり，細胞質においても大規模な増殖巣を作ることは，むしろ腫瘍増殖を妨害することになる。この度のわれわれの腫瘍ウィルスの如き増殖形式は腫瘍の発現のために一つの適当な存在様式と解される。腫瘍性ウィルスの増殖様式自体が一つの研究課題であるとき，この一例の所見は動物および人の腫瘍ウィルス探索上一示唆を与えるものであろう。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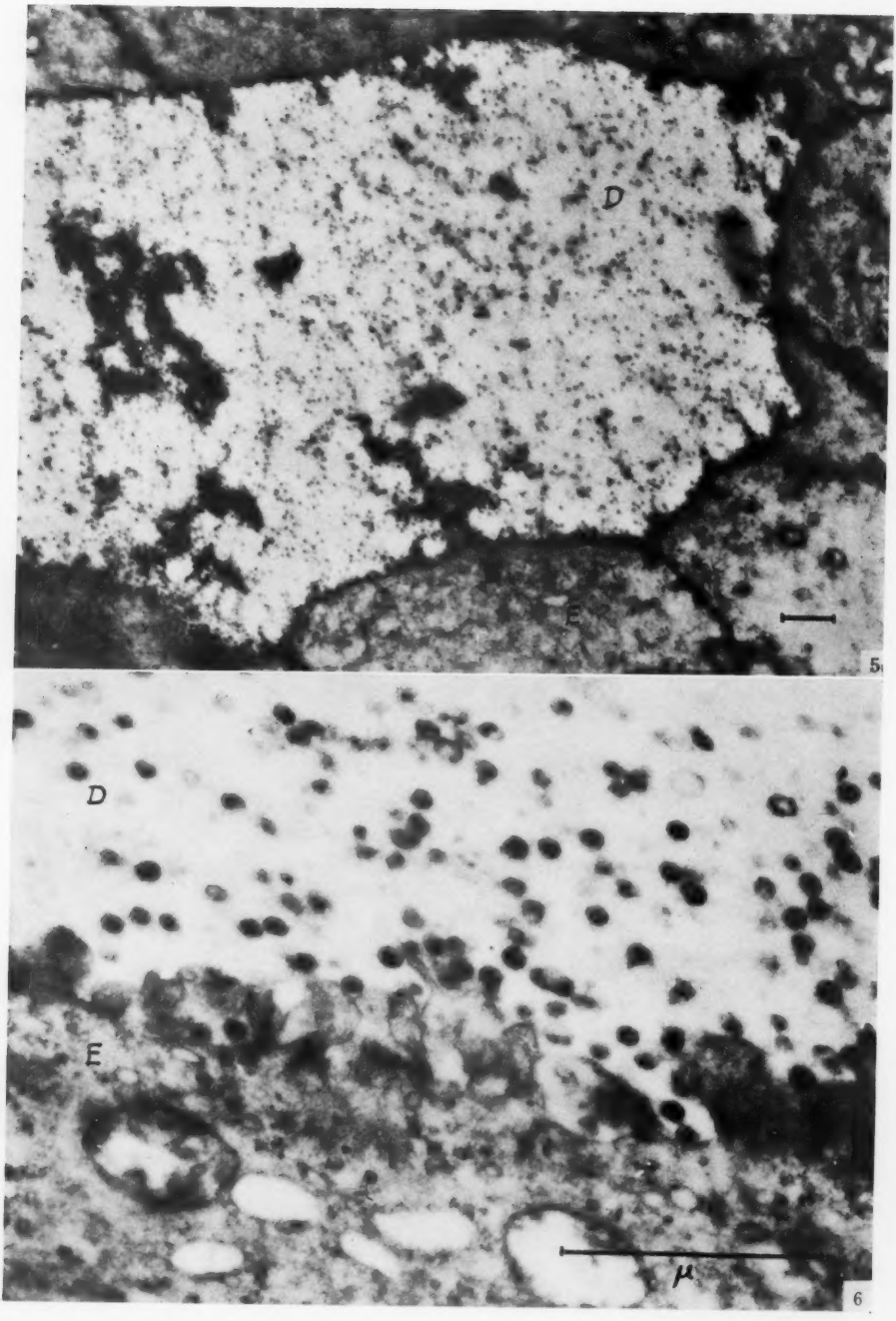
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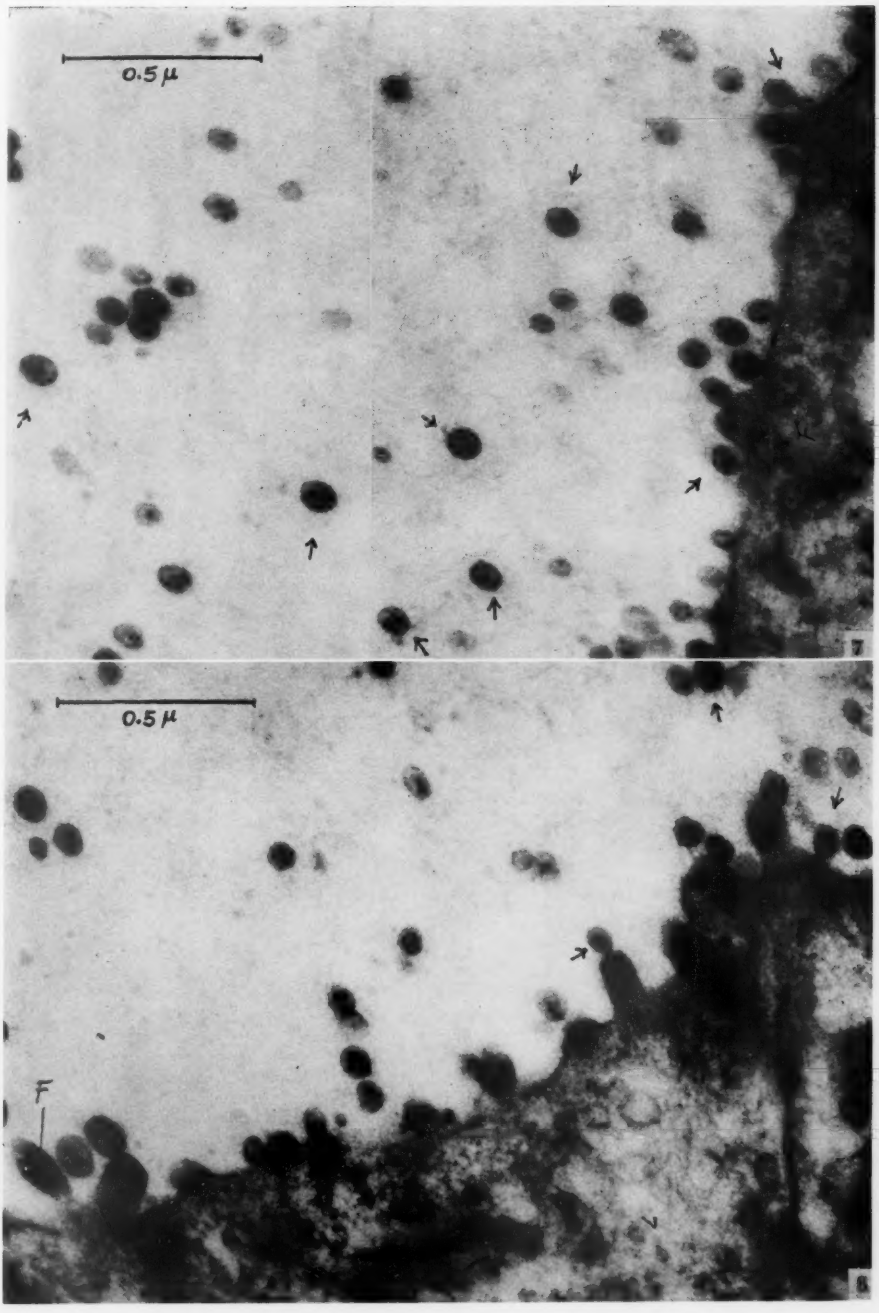
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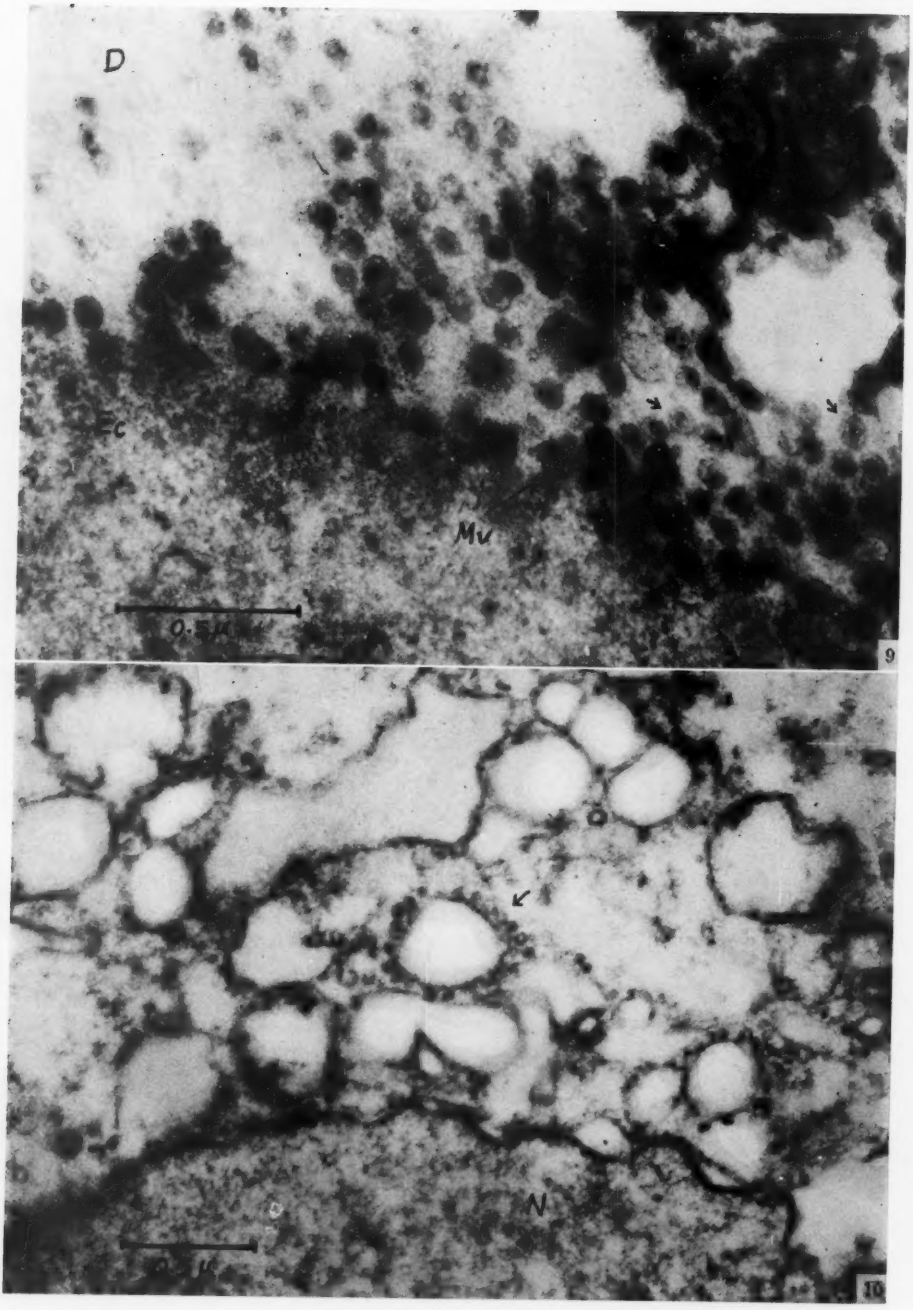
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[GANN, 49, 65~68; March, 1958]

ON THE OCCURRENCE OF OSTEOGENIC FIBROMA ON THE PRE-MAXILLA OF AN INDIAN CAT FISH, WALLAGO ATTU (BLOCH AND SCHNEIDER)

(Plate XVIII)

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The occurrence of spontaneous neoplasm in the jaws of fishes is not very frequent. Schlumberger and Lucke (1948) and Schlumberger (1953) reviewed a few cases of tumors of jaws of fishes arising from the mesenchymal tissues. These tumours have been observed in the following species: fibroma in the lower jaw of a eastern chain pickerel (*Esox niger*) by Nigrelli (1954) and cited by Stolk (1957): a fibrosarcoma in the mandible of a cod (*Pollachius vireus*) by Johnstone (1926); chondromas in the mandible of a barbel (*Barbus fluviatialis*) by Surbeck (1917), and in a minow (*Phoxinus sp.*) by André (1927): osteochondroma in operculum, inferior part of cheekbone and maxilla of a jewl fish (*Hemichromis bimaculatus*) by Nigrelli and Gordon (1946); osteomas in the superior maxilla by Bland-Sutton (1885), in pre-maxilla by Williams (1929) of cod fishes (*Gadus morhus*), in the mandible of a red tai (*Pagrosomus major*) by Kazama (1924). The present paper reports a case of osteogenic fibroma or osteofibroma on the pre-maxilla of an Indian cat fish, *Wallago attu*.

Material and Methods

An adult specimen of *Wallago attu* with a round hard growth on the tip of the upper jaw (Fig. 1) was found along with the other fishes during the netting operation from a perennial tank (area nearly 75 acres with a depth between 12 to 16 feet) in Uttar Pradesh, India. This fish fauna of the tank comprised of *Labeo rohita, Cirrhina maigals, Catla catla, Notopterus notopterus,* species of *Ophicephalus* and other minor fishes. The tank is fed by a permanent channel connected with the nearby river and receives a large volume of rain water during the monsoon months.

The fish in record was 17 lbs. in weight and the body length of the fish was 4 feet. The head of the fish with the growth was properly preserved in 8% formalin.

The material was carefully decalcified and softened in decalcification fluid. Serial sections (10 μ) of the tumour were stained with haematoxylin and eosin

and also according to the Van Giesons methods.

Observations

A very hard prominent knob-like outgrowth, covered with thick skin was present dorsally at the tip of the snout on the pre-maxilla. The outgrowth was roughly oval and trilobed with two dorsal and one ventral lobes. The right lobe (Fig. 2) was large, measuring 3.6 by 3.0 cm. The left lobe was placed immediately after the large one and was partially connected with it and measured 1.9 by 1.6 cm. The small ventral lobe was below the former two and measured 1.0 by 0.9 cm. In the normal position the outgrowth was projected high up with its ventral portion overhanging on the tip of the lower jaw to some extent. The basal portion of the outgrowth was covered by the rough skin which formed more or less a cup like structure. It showed certain interesting features. The anterior part of the cup like structure, adjacent to the upper jaw, was provided with hard pointed normal tooth-like structure. The other three sides of the cup were provided with small warty projections. In the closed condition of the mouth a wide groove was observed below the outgrowth on the upper jaw (Fig. 1).

Skiagram (Fig. 2) shows an intermixture of dense and rarified zone. The tumour was so hard that it was cut open in the middle with the help of a saw for studying the gross internal structures (Fig. 3). The cut surface showed irregular texture. The outgrowth was devoid of any capsule and the skin over it was streched and adherent.

Spherules of multiple bone spicules embedded in a fibrous matrix were observed in the section (Fig. 4). Evidence of calcium deposition here and there was also noted. The laminated appearance of a bony spicules was observed under high power (Fig. 5). A large number of serial sections were examined. but cartilage, multinucleated and spindle shaped cells were absent throughout. The connective tissue structure did not show any sign of malignancy.

Remarks

In the relevant literature data are available concerning the occurrence of mesenchymal tumours like fibroma, fibrosarcoma, chondroma, osteoma, and osteochondroma in the jaws of fishes. No case of osteofibroma from the jaw of the fish has so far been recorded. The occurrence of this type of tumour has been recorded by Montgomery (1927), Phemister and Grimson (1937) and Schlumberger (1946) from the jaws of man. The bore forming tumour in the back ground of fibrous structure may be seen in the conditions such as osteoblastic osteoid tissue forming tumours of bones and osteogenic fibroma or osteofibroma or ossifying fibroma of bone. Jaffe and Mayer (1932) reported an osteoblastic

osteoid tissue forming tumour of metacarpel bone of a 15 years old girl. The tumour was devoid of cartilage and tumour giant cells. Malignant change in connective tissue structure and evidence of the presence of calcium were also not observed. Bergstrand (1930) also noted two osteoblastic osteoid tissue forming tumours. One of them was in finger phalanx and the other was in a metatarsal bone. So the cases described by Jaffe and Mayer and Bergstrand were found to be arising in small bones of cartilagenous origin. Osteoid tissue forming tumours of bone are also commonly found as osteogenic fibroma. This kind of tumour is common in vertebral column though it is also found in flat bone, particularly the mandible and face bones, and they are thought often to represent a solitary focus of fibrous dysplasia. This dysplasia is a highly vascular connective tissue tumour exhibiting osseous metaplasia. The connective tissue cells are described to be small and spindle shaped. There are very often multinuclear cells in proximity to the blood vessels and the trabeculae of the newly formed bone structures. The histological examination of the present tumor shows that the connective tissue is of adult type fibrous tissue. Spindle shaped and multinucleated cells are conspicuously absent from the structure.

Schlumberger (1946) however is of opinion that the arrangement of the new bone in the form of the spicules and the presence of non-ossified deposit of calcium are frequent findings in the so called ossifying fibroma or osteogenic fibroma which has been observed in maxilla and mandible. The present tumour of the fish satisfies these conditions and the place of occurrence of the tumour is also on the premaxilla. On the other hand osteogenic fibromas, arising in both the types of bones, membranous and cartilagenous, have been recorded in the literature. So the tumour under review may be regarded as a case of osteogenic fibroma.

Summary

This communication records osteogenic fibroma from *Wallago attu*. A trilobed hard knob-like big tumour was situated at the tip of the premaxilla and was covered with thick skin. Near the basal portion of the tumour hard pointed teeth-like structures were present at the anterior side while the remaining sides had small warty projections. Irregular structures were observed internally after cutting the tumour in the middle. Spherules of multiple bone spicules, laminated in appearance, embedded in a fibrous matrix were observed on histological examination. Cartilage, multinucleated and spindle shaped cells were absent throughout in the section. The connective tissue did not show any sign of malignancy. Probable histogenesis and comparison of the tumour are discussed.

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Explanation of Figures (Plate XVIII)

Fig. 1. Photograph of the head of *Wallago attu* with the tumour at the tip of the snout.

Fig. 2. Skiagram of the head from below showing right and left lobes of the tumour.

Fig. 3. Photograph of the tumour cut through the middle.

Fig. 4. Photomicrograph of a section of the tumour under low power, ×48, showing spherules of multiple bone spicules.

Fig. 5. The same under high power, ×172, showing laminated bony spicule.

要　　旨

印度産ナマズ１種の骨形成性線維腫について

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印度産ナマズの１種 Wallago attu に見出された骨形生性線維腫の１例を記載し，その組織発生を論議した。

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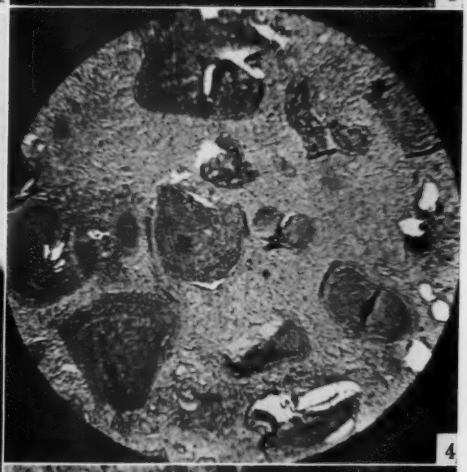
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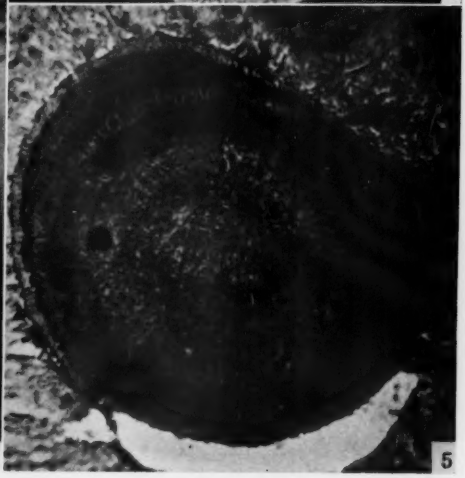
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